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April 30, 2002

**VIA MESSENGER**

Michael L. Mendelsohn
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
US Environmental Protection Agency
Document Processing Desk (7504C)
1921 Jefferson Davis Highway
Crystal Mall 2, Room 266A
Arlington, VA 22202

**RE: 2001 Insect Monitoring Reports EPA Reg. Nos. 524-489; 68467-2; 67979-1; 65268-1; and 29964-3**

Dear Mr. Mendelsohn:

On behalf of the members of the Insect Resistance Management Subcommittee (IRM Subcommittee) of the Agricultural Biotechnology Stewardship Technical Committee, Arent Fox is submitting this letter and seven (7) copies of the enclosed monitoring reports. The IRM Subcommittee members hold the following registrations: Monsanto Company (EPA Reg. No. 524-489); Dow AgroSciences LLC (EPA Reg. No. 68467-2); Syngenta Seeds, Inc. - Field Crops - NAFTA (EPA Reg. No. 67979-1); Syngenta Seeds, Inc. –Vegetables – NAFTA (EPA Reg. No. 65268-1); and Pioneer Hi-Bred International, Inc. (EPA Reg. No. 29964-3). Neither the IRM Subcommittee, nor any of its members, makes any claims of confidentiality with regard to this submission.

On October 15, 2001, the US Environmental Protection Agency (EPA) issued registration amendment letters to the *Bt* field corn registrants. These letters require, *inter alia*, the registrants to submit, by April 30, 2002, a report on results of resistance monitoring and investigations of damage reports. This report is submitted in response to that requirement.

The enclosed submission contains the following third-party reports for European Corn Borer (ECB), Southwestern Corn Borer (SWCB), and Corn Earworm (CEW) insect populations collected during the 2001 growing season:

- Monitoring Bt susceptibility of European Corn Borer to Cry1Ab. B. Siegfried and T. Spencer, University of Nebraska.
- Baseline susceptibility of the European Corn Borer to the Cry1F Bt endotoxin. B. Siegfried and T. Spencer, University of Nebraska.
- Monitoring the susceptibility of the Southwestern Corn Borer, *Diatraea grandiosella*, to *Bacillus thuringiensis* toxin Cry1Ab. Q. Song, C. Luppens, and X. Gan, University of Missouri.
- Monitoring the susceptibility of the Southwestern Corn Borer, *Diatraea grandiosella*, to *Bacillus thuringiensis* toxin Cry1F. Q. Song, C. Luppens, and X. Gan, University of Missouri.
- Monitoring Bt susceptibility of *H. zea* to Cry1Ab. Custom Bio-Products.
- Monitoring Bt susceptibility of *H. zea* to Cry1Fa. Custom Bio-Products.

For the Cry1Ab protein, the reports include the continued monitoring results for ECB and SWCB populations. For both the ECB and SWCB field populations, the results of the dose response tests (i.e. $LC_{50}$, $EC_{50}$) were within the historical ranges established for each pest and no population showed <99% mortality at the diagnostic concentration (the $LC_{99}$ of a susceptible population). Furthermore, all SWCB field populations were more susceptible than the unselected laboratory colony. These data indicate that there has not been a detectable change in Cry1Ab sensitivity among these insect populations compared to the established baseline, and no resistance alleles were detected.

For CEW and Cry1Ab, there were many changes in the monitoring program from previous years owing to a change of cooperating laboratory, to refinement of bioassay methodology and to a change in the toxin source. Additionally, the number of locations from which populations were sampled was small due to lower than expected infestations and to logistical difficulties. These changes and the small sample size make a reliable comparison with previous baseline data difficult, and the data should therefore be treated as establishing a new baseline. However, the variability in response among populations ($LC_{50}$s from 1.37 to 4.98 ng / $cm^2$ diet) is within the range expected from published literature (e.g. 16-fold reported by Stone, T. B. and S. R. Sims. 1993. J. Econ Entomol 86: 989-994).

For the Cry1F protein, the reports include data to both establish a baseline susceptibility level and initiate the monitoring program for ECB, SWCB and CEW populations before the commercial deployment of this protein in corn. ECB and SWCB results showed no significant changes in the susceptibility to Cry1F between 2000 and 2001. For CEW, growth inhibition and mortality data indicate some variability among the populations; however this variability is similar to that reported for other proteins. As described above, the number of CEW populations available for testing was limited in 2001. ABSTC will continue to work with our collaborators to enhance the CEW field collection program.

The registrants received no legitimate reports of unacceptable product performance for control of the target pests; therefore, no reports of such investigations are included in this submission.

If the Agency has any technical questions or concerns with the reports or this submission, please immediately contact Nicholas P. Storer (IRM Subcommittee Chair, tel. 317-337-5138).

Sincerely,

Stanley H. Abramson
Authorized Representative of the
Agricultural Biotechnology Stewardship
Technical Committee

Enclosures (6)

# MONITORING BT SUCSEPTIBILITY OF EUROPEAN CORN BORER TO CRY1AB

## 2001 Data Summary

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University of Nebraska
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**INTRODUCTION:** Widespread exposure of European corn borer (ECB) populations to transgenic corn expressing the Cry1Ab toxin from *Bacillus thuringiensis* may result in selection for resistance resulting in elevated $LC_{50}$'s and increased survival at diagnostic Bt concentrations. Because of similarity in the technology within the seed industry, a standardized monitoring program involving $LC_{50}$ determinations and diagnostic bioassays was conducted in 2001. Collections for the 2001 monitoring effort were coordinated by members of the Agricultural Biotechnology Stewardship Technical Committee (ABSTC). In 2000, collections sites were chosen based on 2000 market penetration of Bt corn. This information was derived from sales records provided ABSTC member companies. Additionally, information based on insecticide use was used to assist in identification of areas at high risk of resistance development. Three broad geographic areas were chosen based on the above criteria: 1) Southwestern Minnesota, Eastern South Dakota, Southeast North Dakota, and Northwest Iowa; 2) Southwest Kansas and the Texas, Oklahoma Panhandle; 3) Central to Southeastern Iowa and North Central Illinois. A detailed description of the criteria used to establish collection areas is provided in the ABSTC Monitoring Plan submitted to EPA on March 31, 2000.

**OBJECTIVE:** Compare levels of susceptibility among geographically distinct ECB populations using dose-response regressions and diagnostic bioassays, emphasizing collections in areas where transgenic Bt corn is likely to obtain a high market share.

**METHODS:**

**Field Collections and Rearing:**

Field collections consisted of either adults obtained from light traps or from sweep net samples, second generation egg masses and diapausing larvae (Table 1). A total of 13 populations were sampled from the various regions identified above. Two additional populations were obtained from Nebraska. A summary of the populations sampled is provided in Table 1.

Rearing procedures for ECB have been widely reported and are based on those developed at the USDA Lab in Ankeny, IA (Guthrie et al. 1965). Larvae were reared at 27ºC in 24 h light and 80% RH on wheat germ based diet (Lewis and Lynch 1969). At

pupation, insects are moved to mating cages where adults are maintained with 8 h scotophase at 18.3ºC and 16 h photophase at 27ºC with RH at 80%. Egg masses from the mated females are collected and held within plastic Petri dishes provided with filter paper moistened with sterile water to prevent desiccation, and incubated at 27ºC until hatching.

Table 1. 2001 ECB collections used for bioassay of Cry1Ab susceptibility.

| Colony/Location | Life Stage at Collection | Number Collected | Generation Bioassayed[1] |
|---|---|---|---|
| Aurora, NE | Moths | 473 | F1 |
| Champaign Co., IL | Egg Mass (EM) | 168 | F1 |
| Clinton Co., IA | EM | 64 | F1 |
| Dakota Co., MN | Moths | ≈260 | F0 |
| DeKalb Co., IL | EM | 82 | F1 |
| Goodhue Co., MN | Moths | 357 | F1 |
| Henry Co., IA | EM | 97 | F1 |
| Mead, NE | Moths | 455 | F0 |
| Warren Co., IL | EM | 97 | F1 |
| Waterman Co., IL | EM | 139 | F1 |
| Garden City, KS | Larvae & pupae | 14 | F2 |
| Garden City, KS | Larvae | 100 | In diapause* |
| Lincoln, Co., SD | Larvae | 119 | In diapause* |
| Forestburg, SD | Larvae | 108 | In diapause* |
| Kandiyohi Co., MN | Larvae | 126 | In diapause** |

[1] F0 = progeny from field collected adults bioassayed directly; F1 = field collections reared in laboratory for one generation before bioassays; F2 = 2 generations of lab rearing before bioassays

* Insects still in diapause and not yet assayed

**Bioassays**:

*1. Determination of $LC_{50}$'s:* Bioassay of neonate ECB larvae involved exposure to Bt solutions applied to the surface of single wells of artificial diet. We attempted to utilize progeny ($F_1$) obtained directly from field collected insects. However, in some instances, we utilized insects from both the $F_1$ and $F_2$ generation. Bioassays were performed in 128 well trays (each well 16 mm diam. x 16 mm height; CD International, Pitman, NJ). Dilutions of Bt were prepared in 0.1%Triton-X 100 to obtain uniform spreading of Bt solution on the diet surface.

Individual neonate larvae (less than 24 h after hatching) were placed in wells, a nd mortality and combined larval weight recorded 7 days later. Control treatments consisted of wells treated with 0.1% Triton-X 100. When recording mortality, larvae that had not grown beyond first instar (i.e., $\leq$0.1 mg) were considered to be dead. Bioassays were conducted in duplicate on three different dates and included at least five Bt concentrations that produced mortality >0 but <100%. Data were analyzed by probit analysis (Finney 1971, LeOra Software 1987) to determine lethal concentrations. Observed mortality was corrected for mortality in control treatments, and lethal concentrations with 95% fiducial limits were calculated. Larval weights were transformed to % growth inhibition relative to the controls and these data were analyzed by non-linear regression (SAS Institute Inc. 1988).

The protein used for bioassays consisted of a formulated Cry1Ab (CellCap®) provided by Dow/Mycogen (San Diego, CA; Lot# MR818 571-1457; 33.18 mg Cry1Ab/g formulation), and is the same source used in 2000 bioassays to determine Cry1Ab susceptibility.

2. *Discriminating Concentration Bioassays:* The original $LC_{99}$ of the purified Cry1Ab Bt toxin was estimated from baseline susceptibility studies conducted on 13 populations in 1995 and used as a discriminating concentration (Marçon et al. 2000). In 2001, we utilized the same concentration as developed in 2000 bioassays.

Bioassays with discriminating concentrations employed the same techniques described above. Egg masses collected during a given 24 hr period were held in plastic Petri dishes, provided with filter paper moistened with sterile water to prevent desiccation, and incubated at 27ºC until hatching. Neonate larvae were selected at random and placed in individual wells treated as described above at a concentration corresponding to the estimated $LC_{99}$. A total of 560 individual larvae were sampled from each collection. The proportion of surviving larvae exhibiting weight of 0.1 mg was recorded 7 days later.

**Results and Discussion**: Results from concentration-mortality tests using Cry1Ab on the populations tested during 2000 are presented in Table 3. Although there was significant variation in $LC_{50}$ values as indicated by non-overlapping confidence intervals, the variability was not different from that observed in baseline tests conducted from 1995 to 2000 (Fig. 1). Additionally, the mortality observed at a diagnostic concentration corresponding to an overall $LC_{99}$ derived from baseline data, was nearly 100% in all the populations tested. These results parallel those reported from 1996-2000 where mortality in all populations examined was greater than 99 percent. None of the populations exhibited less than 99% mortality indicating that all populations remain susceptible to the Cry1Ab toxin.

A similar pattern of susceptibility was observed from $EC_{50}$ values derived from non-linear regression of larval growth inhibition data (Fig. 2). As described previously (Marçon et al. 1999), growth inhibition assays are more sensitive in that toxicity can be detected at

lower toxin concentrations although a similar level of variability (Fig. 1) is observed among populations.

Table 3. Susceptibility of European corn borer neonate larvae to the Cry1Ab Bt toxin.

| Collection Site | Slope ± SE | $LC_{50}$* (95% FL) | $LC_{90}$* (95% FL) | % Mortality** |
|---|---|---|---|---|
| Aurora, NE | 1.47 ± 0.14 | 0.14 ( 0.06 – 0.24 ) | 1.86 ( 0.98 – 6.28 ) | 100.00% |
| Champaign County, | 2.21 ± 0.22 | 0.17 ( 0.12 – 0.22 ) | 0.92 ( 0.63 – 1.66 ) | 99.70% |
| Clinton County, IA | 2.07 ± 0.16 | 0.20 ( 0.14 – 0.25 ) | 1.22 ( 0.88 – 1.98 ) | 99.70% |
| Dakota County, MN | 2.75 ± 0.31 | 0.25 ( 0.14 – 0.35 ) | 1.01 ( 0.68 – 2.56 ) | 99.85% |
| DeKalb County, IL | 2.91 ± 0.30 | 0.15 ( 0.11 – 0.19 ) | 0.54 ( 0.37 – 1.01 ) | 100.00% |
| Goodhue County, MN | 1.81 ± 0.15 | 0.22 ( 0.11 – 0.36 ) | 1.79 ( 1.00 – 5.42 ) | 100.00% |
| Henry County, IA | 2.12 + 0.24 | 0.28 ( 0.15 – 0.40 ) | 1.66 ( 1.09 – 3.96 ) | 100.00% |
| Mead, NE | 2.05 ± 0.19 | 0.32 ( 0.25 – 0.39 ) | 2.03 ( 1.55 – 2.89 ) | 100.00% |
| Warren County, IL | 2.11 ± 0.23 | 0.20 ( 0.10 – 0.30 ) | 1.22 ( 0.79 – 2.91 ) | 99.70% |
| Waterman, IL | 2.81 ± 0.30 | 0.37 ( 0.31 – 0.44 ) | 1.44 ( 1.13 – 2.03 ) | 100.00% |

* ng Cry1Ab/cm$^2$

¨ Refers to % mortality (i.e., mortality and stunting) at diagnostic concentration (10 ng/cm$^2$).

† IP=In progress; Two additional colonies have required additional rearing in order to obtain enough individuals to complete bioassays, and results will be appended at a later date.

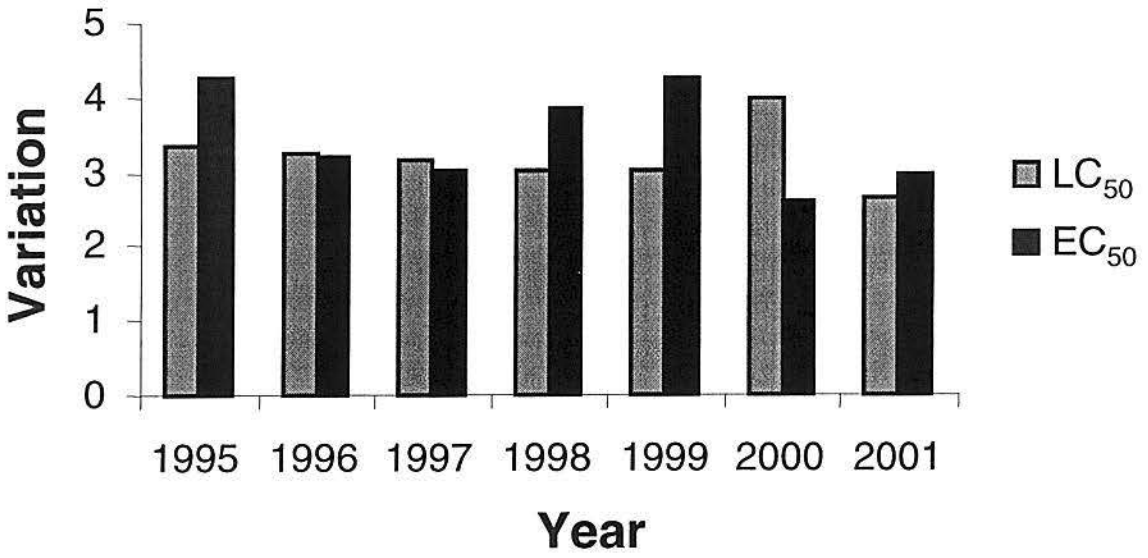


*Figure 1. Variability in response to Cry1Ab among ECB field populations from 1995-2000. Variation assessed by ratio of high:low $LC_{50}$ and $EC_{50}$ for each year of monitoring.*

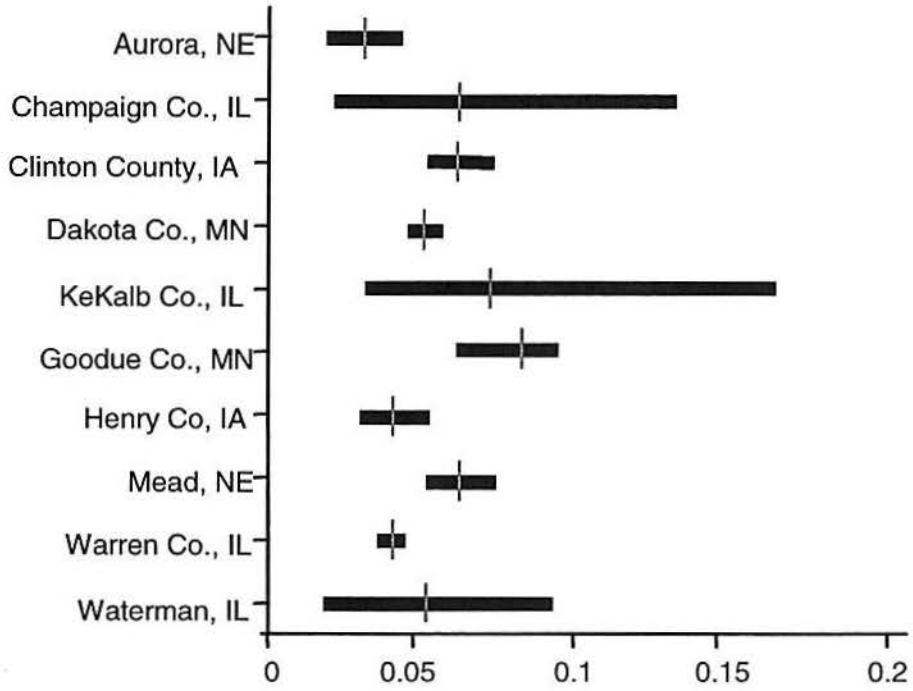


*Figure 2. $EC_{50}$'s of European corn borer neonate larvae to the Cry1Ab Bt toxin.*

Based on the results of dose-mortality assays and diagnostic bioassays employing the Cry1Ab toxin, it is unlikely that the small differences in response are the result of prior selection and are more likely to have resulted from natural variability in susceptibility among ECB populations (Robertson et al. 1995, Marçon et al. 1999). These results in combination indicate that in the five years of Bt corn availability, there has not been a detectable change in Cry1Ab susceptibility among ECB populations as a result of selective pressures resulting from the introduction of transgenic corn.

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Marçon, P.R.G.C., L.J. Young, K. Steffey, and B.D. Siegfried. 1999. Baseline susceptibility of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 92: 2799-285.

Marçon, P.R.G.C., B.D. Siegfried, T. Spencer and W.D. Hutchison. 2000. Development of diagnostic concentrations for monitorin Bacillus thuringiensis resistance in European corn borer (Lepidoptera: Crambidae) J. Econ. Entomol. 93: 925-930.

Robertson, J. L., H. K. Preisler, S. S. NG, L. A. Hickle, and W. D. Gelernter. 1995. Natural variation: a complicating factor in bioassays with chemical and microbial pesticides. J. Econ. Entomol. 88: 1-10.

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# BASELINE SUSCEPTIBILITY OF THE EUROPEAN CORN BORER TO THE CRY1F Bt ENDOTOXIN

## 2000-2001 Data Summary

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**Introduction:** The ability to detect insecticide resistance in pest populations is necessary to: 1) determine whether control failures are due to the presence of resistant insects some other factor that affects product performance; 2) assess the extent and distribution of resistant populations; and 3) test the effectiveness of management programs designed to reduce the frequency of resistant individuals. In anticipation of the introduction of Cry1F expressing corn hybrids, baseline susceptibility of the primary target pest, the European corn borer (ECB) was initiated in 1999 and continued in 2000-2001. Additionally, candidate diagnostic concentrations were tested based on calculated $LC_{99}$ and $EC_{99}$ values from initial baseline assessments.

**Methods:** Methods for rearing and bioassay of the Cry1F toxin against neonate ECB larvae are identical to those previously reported in the 2000 and 2001 annual reports of Cry1Ab susceptibility. Field collections consisted of either adults obtained from light traps or from sweep net samples, second generation egg masses and diapausing larvae. The protein used for bioassays consisted of chromatographically purified and proteolytically truncated Cry1F Bt toxin (provided by Dow AgroSciences; Indianapolis, IN).

Two candidate diagnostic concentrations were also tested in 2000 and 2001 that corresponded to the upper limit of the 95% fiducial limit of the $LC_{99}$ and $EC_{99}$ derived from initial baseline assessments conducted in 1999 and similar to the methods used to derive a diagnostic concentration for Cry1Ab (Marçon et al. 2000).

**Results and Discussion**: Results from concentration-mortality tests using Cry1F on the populations collected during 2000 and 2001 are presented in Table 1 and 2, respectively. Although there was significant variation in $LC_{50}$ values as indicated by non-overlapping confidence intervals, the variability observed was similar to other toxin Bt toxins using identical procedures.

A similar pattern of susceptibility was observed from $EC_{50}$ values derived from non-linear regression of larval growth inhibition data (Fig. 1). As described (Marçon et al. 1999), growth inhibition assays are more sensitive in that toxicity can be detected at lower concentrations, although the variability among populations is comparable to that observed for mortality.

**Table 1.** 2000 Susceptibility of European corn borer neonate larvae to the Cry1F Bt toxin.

| Population | N | Slope: | $LC_{50}$*(95% FL) | $LC_{90}$*(95%FL) |
|---|---|---|---|---|
| Beadle Co, SD | 766 | 4.54 ± 0.62 | 4.11 (3.51 - 4.62) | 7.87 (6.9 - 9.54) |
| Moody Co, SD | 766 | 2.64 ± 0.28 | 2.35 (1.86 - 2.84) | 7.21 (5.9 - 9.33) |
| Nobles Co, MN | 767 | 2.0 ± 0.16 | 2.37 (1.51 - 3.33) | 10.37 (7.12 - 18.19) |
| Saunders Co, NE | 768 | 2.59 ± 0.25 | 3.91 (3.2 - 4.61) | 12.18 (10.17 - 15.3) |
| Hamilton Co, NE | 768 | 2.41 ± 0.22 | 2.88 (1.94 - 3.84) | 9.8 (7.25 - 15.2) |
| Scott Co, KS | 768 | 2.49 ± 0.18 | 2.55 (2.22 - 2.91) | 8.33 (7.0 - 10.34) |
| Cent. Finney Co, KS | 764 | 1.96 ± 0.16 | 4.12 (2.99 - 5.38) | 18.59 (13.53 - 29.07) |
| So. Finney Co, KS | 768 | 3.2 ± 0.31 | 4.96 (4.25 - 5.67) | 12.48 (10.66 - 15.29) |
| Marion Co, IA | 753 | 2.12 ± 0.17 | 2.57 (1.81 - 3.41) | 10.29 (7.37 - 16.82) |
| Story Co, IA | 1151 | 2.8 ± 0.34 | 3.17 (1.95 - 4.19) | 9.12 (6.95 - 14.48) |
| Polk/Tama Co, IA | 1533 | 2.33 ± 0.2 | 4.74 (3.3 - 6.26) | 16.83 (12.21 - 27.62) |
| Sioux Co, IA | 767 | 2.38 ± 0.2 | 3.23 (2.7 - 3.79) | 11.16 (9.29 - 14.03) |
| Linn Co, IA | 384 | 2.86 ± 0.26 | 3.04 (2.40 - 3.70) | 8.51 (6.80 - 11.61) |
| Warren Co, IL | 768 | 2.24 ± 0.14 | 3.81 (3.34 - 4.33) | 14.25 (11.84 - 17.84) |
| Henderson Co, IL | 128 | 2.86 ± 0.22 | 6.26 (5.15 - 7.46) | 17.55 (14.02 - 23.96) |

* ng Cry1F/cm

**Table 2.** 2001 Susceptibility of European corn borer neonate larvae to the Cry1F Bt toxin.

| Population | N | Slope ± SE | $LC_{50}$(95% CL) | $LC_{95}$(95% CL) |
|---|---|---|---|---|
| Aurora, NE | 764 | 2.18 ± 0.17 | 3.68 ( 2.69 – 4.77 ) | 20.93 ( 14.88 – 34.53 ) |
| Champaign County, IL | 764 | 2.88 ± 0.33 | 1.23 ( 0.96 – 1.49 ) | 4.59 ( 3.74 – 6.09 ) |
| Clinton County, IA | 761 | 2.32 ± 0.25 | 2.26 ( 1.38 – 3.06 ) | 11.55 ( 8.14 – 21.40 ) |
| Dakota County, MN | 768 | 2.59 ± 0.24 | 2.38 ( 1.99 – 2.76 ) | 10.28 ( 8.37 – 13.53 ) |
| DeKalb County, IL | 761 | 2.07 ± 0.18 | 1.47 ( 1.00 – 1.95 ) | 9.17 ( 6.49 – 15.35 ) |
| Goodhue County, MN | 760 | 1.67 ± 0.13 | 2.20 ( 1.03 – 3.65 ) | 21.25 ( 10.97 – 83.89 ) |
| Henry County, IA | 765 | 2.17 ± 0.20 | 1.98 ( 1.57 – 2.40 ) | 11.36 ( 8.99 – 15.47 ) |
| Mead, NE | 762 | 1.70 ± 0.16 | 2.28 ( 1.70 – 2.90 ) | 21.22 ( 15.90 – 13.07 ) |
| Warren County, IL | 764 | 1.95 ± 0.19 | 1.42 ( 0.79 – 2.08 ) | 9.90 ( 6.47 – 20.24 ) |
| Waterman, IL | 768 | 2.52 ± 0.20 | 2.92 ( 2.17 – 3.71 ) | 13.14 ( 9.44 – 22.12 ) |

* ng Cry1F/cm

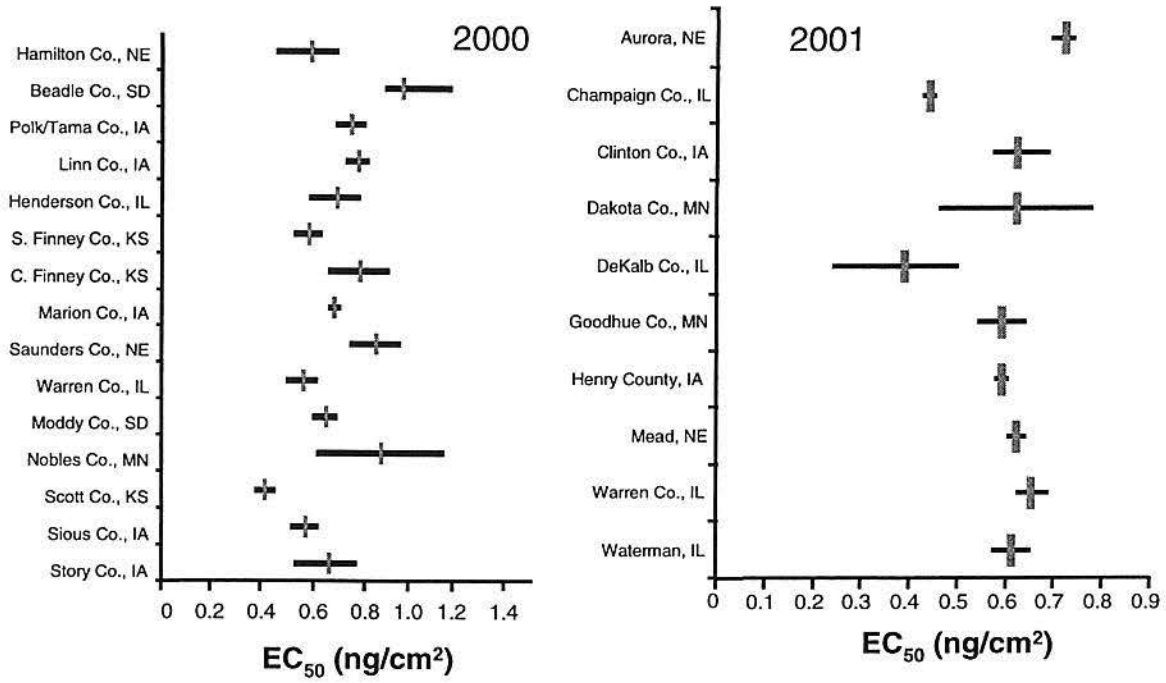


**Figure 1.** $EC_{50}$'s (Concentration that causes 50% growth inhibition) of European corn borer neonate larvae exposed to the Cry1F Bt toxin. Horizontal bars indicate 95% confidence intervals.

Based on the results of dose-mortality assays, it is unlikely that the small differences in response are the result of prior selection and are more likely to have resulted from natural variability in susceptibility among ECB populations (Robertson et al. 1995, Marçon et al. 1999).

Results of tests to validate diagnostic concentrations are summarized in Tables 3 and 4. In 2000 (Table 2) both the $LC_{99}$ and $EC_{99}$ were tested to determine whether the calculated values correspond to levels or mortality and growth inhibition observed among field populations. In both years, the resulting mortality and growth inhibition were similar to the expected values. However, there were two populations in 2000 that exhibited growth inhibition less than 99% (Beadle Co., SD and Central Finney Co., KS), and one of these (Finney Co., KS) also exhibited mortality less than 99% at the $LC_{99}$. In 2001, both populations from Nebraska exhibited mortality less than 99%. These results indicate that the calculated values should be adjusted somewhat higher in order to reliably achieve mortality that is $\geq$ 99% in all field populations.

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Marçon, P.R.G.C., B.D. Siegfried, T. Spencer and W.D. Hutchison. 2000. Development of diagnostic concentrations for monitorin Bacillus thuringiensis resistance in European corn borer (Lepidoptera: Crambidae) J. Econ. Entomol. 93: 925-930.

| **Table 3.** 2000 Cry1F Diagnostic Bioassays | | $EC_{99}$[1] | | $LC_{99}$[2] | |
|---|---|---|---|---|---|
| **Colony** | **N** | **%Mortality[3]** | **% Inhibition** | **N** | **% Mortality[3]** |
| Beadle Co, SD | 447 | 65.10 | 93.60 | 544 | 99.63 |
| Moody Co, SD | 671 | 92.40 | 99.37 | 336 | 99.40 |
| Nobles Co, MN | 333 | 92.79 | 99.81 | 336 | 100.00 |
| Saunders Co, NE | 334 | 86.83 | 99.27 | 335 | 100.00 |
| Hamilton Co, NE | 333 | 98.50 | 99.88 | 336 | 100.00 |
| Scott Co, KS | 560 | 92.68 | 99.77 | 336 | 99.40 |
| Cent. Finney Co, KS | 447 | 63.53 | 94.05 | 448 | 98.66* |
| So. Finney Co, KS (B) | 328 | 93.29 | 99.77 | 328 | 100.00 |
| Marion Co, IA | 334 | 91.92 | 99.69 | 333 | 100.00 |
| Story Co, IA | 336 | 97.62 | 99.93 | 336 | 100.00 |
| Polk/Tama Co's, IA | 448 | 95.76 | 99.88 | 334 | 99.10 |
| Sioux Co, IA | 335 | 96.12 | 99.94 | 336 | 99.70 |
| Linn Co, IA | 223 | 90.58 | 99.62 | 336 | 99.70 |
| Warren Co, IL | 332 | 97.59 | 99.73 | 335 | 100.00 |
| Henderson Co, IL | 335 | 95.82 | 99.88 | 335 | 99.10 |
| **Cumulative** | **3678** | **90.86** | **98.95** | **3457** | **99.54** |

[1] Calculated from 1999 baseline data as 15 ng/cm$^2$

[2] Calculated from 1999 baseline data as 60 ng/cm$^2$

[3] Mortality refers to individuals that were either dead or did not grow beyond 0. 1 mg.

| **Table 4.** 2001 Cry1F Diagnostic Bioassays | **N** | **% Mortality[1] at $LC_{99}$** |
|---|---|---|
| Aurora, NE | 663 | 98.64 |
| Champaign Co., IL | 670 | 100.00 |
| Clinton Co., IA | 781 | 100.00 |
| Dakota Co., MN | 671 | 100.00 |
| DeKalb Co., IL | 666 | 100.00 |
| Goodhue Co., MN | 668 | 99.70 |
| Henry Co., IA | 668 | 99.25 |
| Mead, NE | 671 | 97.47 |
| Warren Co., IL | 783 | 100.00 |
| Waterman, IL | 665 | 99.85 |
| **Cumulative** | **6906** | **99.51** |

[1] Mortality refers to individuals that were either dead or did not grow beyond 0. 1 mg; $LC_{99}$ Calculated from 1999 baseline data as 60 ng/cm$^2$

# MONITORING THE SUSCEPTIBILITY OF THE SOUTHWESTERN CORN BORER, *DIATRAEA GRANDIOSELLA*, TO *BACILLUS THURINGIENSIS* toxin Cry1Ab

## 2001 Data Summary

Qisheng Song, Chris Luppens, and Xingsheng Gan
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Columbia, MO 65211

(January 22, 2002)

## INTRODUCTION

This is a continuous effort to monitor the susceptibility of the Southwestern corer borer (SWCB), *Diatraea grandiosella*, to *Bacillus thuringiensis* (Bt.) toxins Cry1Ab.

In this report, six field populations of the SWCB were collected from two regions with high Bt-corn penetration rate and bioassayed to determine a baseline susceptibility (larval mortality, larval growth inhibition and response to diagnostic dose) to Bt. toxins Cry1Ab.

## EXPERIMENT PROCEDURES

### a. Field SWCB sample collection

The SWCB field samples were collected from the same locations as we did last year (Table 1). In brief, two sample collection regions were selected based on the high Bt-corn penetration rate and/or a history of insecticide application in refuge areas. The region one includes southwest Kansas and the panhandle area of Oklahoma and Texas with both a high rate of Bt-corn and a history of insecticide applications. Two field

populations were collected from this region. Diapause larvae (157) were collected from Garden City of KS by Larry Buschman of KSU and second flight Female adults (107) were blacklight-trapped from Hale Co. of TX by Pat Porter of TAM. There was no sample available from St. John (KS), presumably due to extremely cold winter last year (personal communication with Larry Buschman). The Region two includes the areas of Missouri Bootheel, Western Kentucky, Western Tennessee, and Extreme Southern Illinois with a relatively high penetration of Bt. corn. Four field populations were collected from the region two (Michael Boyd of MU at Delta Center collected one population of diapause larvae from Delta center, MO; My student Chris Luppens and I collected three field populations by blacklight trapping, one each from MO-Dexter, TE-Milan, and KY-Princeton) (Table 1).

**b. Rearing of SWCB**

Larvae generated from the field-collected populations and from a MS-laboratory colony were maintained on a BioServe artificial diet in clear plastic cups (20 ml) using established laboratory procedures (Chippendale and Cassatt, 1985). Larvae are incubated at $30^0$C and 16 hr L : 8 hr D, whereas eggs, pupae, and adults are incubated at $25^0$C and 13 hr L : 11 hr D.

**c. CrylAb**

Cry1Ab protein was provided by Monsanto last year (2000), aliquoted and stored at $-80^0$C until use.

**d. Susceptibility ($LC_{50}$ and $EC_{50}$) of SWCB neonate larvae to Cry1Ab toxin**

*Larval mortality*

All experimental procedures were identical to that of last year. In brief, a larval feeding bioassay was used. Cry1Ab toxin was diluted in a 50 ml centrifuge tube containing 1 ml of distilled water to make a serial of desired concentration, and the

solution was incorporated into 40 ml of artificial diet (kept at $55^0$C water bath) by inverting and shaking the tube. The diet containing the indicated dose of Cry1Ab was then pipetted into a 128-cavity tray (BIO BA 128 bioassay tray, C-D International, Ocean City, NJ) with 1 ml of diet/cavity. The diet-Cry1Ab containing tray was either assayed shortly after cool down or kept at $4^0$C for < 48 h before use. Newly hatched larvae (<24 h) were transferred individually to each cavity containing 1 ml of diet with or without the indicated concentration of Cry1Ab. Each concentration was replicated four times (25 larvae replicate$^{-1}$) using larvae from different batches of the $F_2$ or $F_3$ generations (the number of $F_1$ generation larvae were usually not enough for the assays in such a scale). Larval mortality was observed at 7 and 14 days after treatment (DAT). Larvae were considered dead if they do not move when they were probed using a camel hairbrush or when they were still alive but remained at 1$^{st}$ instar stage (these larvae will be dead in the field conditions).

Six or seven consecutive concentrations of each toxin, including the control, were used for probit analyses to determine the $LC_{50}$ and $LC_{95}$ values on 7 and 14 DAT. These concentrations produce larval mortality of >0% but <100%. Probit analyses were conducted, using SAS program.

*Larval growth inhibition*

Six concentrations of Cry1Ab were used in bioassay. Each concentration was replicated 4 times (25 larvae replicate$^{-1}$) from different batches as described above. The mean weight of newly hatch larvae (initial weight) was determined by weighing a group of 25 and the mean weight of those surviving larvae at each tested dose was determined at 14 DAT.

Regression analyses (log concentration vs. weight gain inhibition [%]), are used to determine the $EC_{50}$ and $EC_{95}$ values at 14 DAT. Weight gain inhibition represents the relative differences in weight gain of the treated larvae compared to that of the control larvae. The following equation is used to correct for initial weight: $I = \{(C - T) \div (C - B)\} \times 100\%$, where I = weight gain inhibition (%); C = mean weight of control

larva (mg larva$^{-1}$); T = mean weight of surviving larvae from the treated diets (mg larva$^{-1}$); and B = mean initial larval weight (mg larva$^{-1}$).

*Efficacy of the diagnostic concentration against the laboratory-adapted and field-collected populations* of *Diatraea grandiosella*

The diagnostic concentration was estimated from the $LC_{99}$ value at 7 and 14 DAT of the MO-Laboratory population previously determined because this population has been found to be the least susceptible to Cry1Ab protein of the populations tested (Trisyono and Chippendale, 1999 data). 6 field populations of SWCB were used to test the efficacy of the diagnostic concentration (35 µg/g diet for 7 DAT, 5 µg/g diet for 14 DAT) in determining the susceptibility to Cry1Ab.

Two hundred of newly hatched larvae from $F_2$ or $F_3$ of each field population were transferred individually in to the 128-cavity tray containing 1 ml of control diet or the diet with the diagnostic concentration of Cry1Ab. On the 7 and 14 DAT, larval mortality was recorded as described above.

**Results and Discussions**

Bioassay of the susceptibility of neonate SWCB larvae to Cry1Ab toxin had been summarized in Table 2 and 3. This was the continuation of the previous year's monitoring effort to detect any significant changes in the susceptibility of SWCB to Cry1Ab. All bioassays were performed in128-cavaty tray (C-D International) as we did last year (2000) and at least 100 larvae were tested for each concentration of Cry1Ab.

Based on the $LC_{50}$ and $LC_{95}$ values (Table 2) and $EC_{50}$ and $EC_{95}$ value (Table 3), significant variation was observed among geographically distinct populations of SWCB in their susceptibility to Cry1Ab toxin. However, these variations are within the range of 1998 and 1999 data (Trisyono and Chippendale, 1999 report) and 2000 data (Song et al.,

2000). All field-collected populations were more susceptible to Cry1Ab toxin than the MS-lab colony (MS-lab colony was reintroduced from Frank Davis's lab in Fall of 2000).

The diagnostic concentration was estimated from the $LC_{99}$ value of MO-lab colony, the least susceptible colony determined previously by Trisyono and Chippendale (1999). It's not surprised that none of the larvae survived the diagnostic concentration of Cry1Ab (Table 2) since the $LC_{99}$ value of MO-lab colony (1999 data) was much higher than that of the 6 field populations tested in 2001.

Bioassay data suggest that no sign of significant level of changes in the susceptibility of SWCB to Cry1Ab has been developed in the field populations tested so far when compared to the previous data (Fig. 1).

**REFERENCES**

Chippendale GM and Cassatt KL (1985) *Diatraea grandiosella,* in *Handbook of Insect Rearing, vol 2,* ed Singh P and Moore RF, Elsevier Science Publishers, Amsterdam, pp 257-264.

Trisyono A and Chippendale M (1999) Monitoring the susceptibility of southwestern corn borer, Diatraea grandiosella, to Bacillus thuringiensis. Assay report.

Song Q, Luppens C. and Gan X (2000) Monitor the susceptibility of the Southwestern corer borer (SWCB), *Diatraea grandiosella*, to *Bacillus thuringiensis* (Bt.) toxins Cry1Ab. Assay report.

Table 1. 2001 SWCB collections used for bioassay of Cry1F susceptibility.

| Region* | State | County(Bt corn Penetration rate %) | Designation | Starting population (#) |
|---|---|---|---|---|
| 4 | Missouri | New Madrid (30-39) | Delta Center | 156 DL |
| | Missouri | Stoddard (20-29) | Dexter | 104 FA ($F_2$) |
| | Kentucky | Caldwell (?) | Princeton | 124 FA ($F_2$) |
| | Tennessee | Gibson (20-29) | Milan | 89 FA ($F_2$) |
| 2 | Kansas | Stafford (>50%) | St. John | No sample for 2001 |
| | Kansas | Finney (10-19) | Garden City | 147 DL |
| | Texas | Hale (10-19) | Plainview | 103 FA ($F_2$) |
| Lab colony | Mississippi | Laboratory | MS-Lab | >1,000 EM |

* These two regions were selected for SWCB sample collection by ABSTC in March, 2000.
EM= egg masses; DL=diapause larvae; $F_2$= second fly adults.

Table 2. Susceptibility of SWCB neonate larvae to the Cry1F Bt toxin.

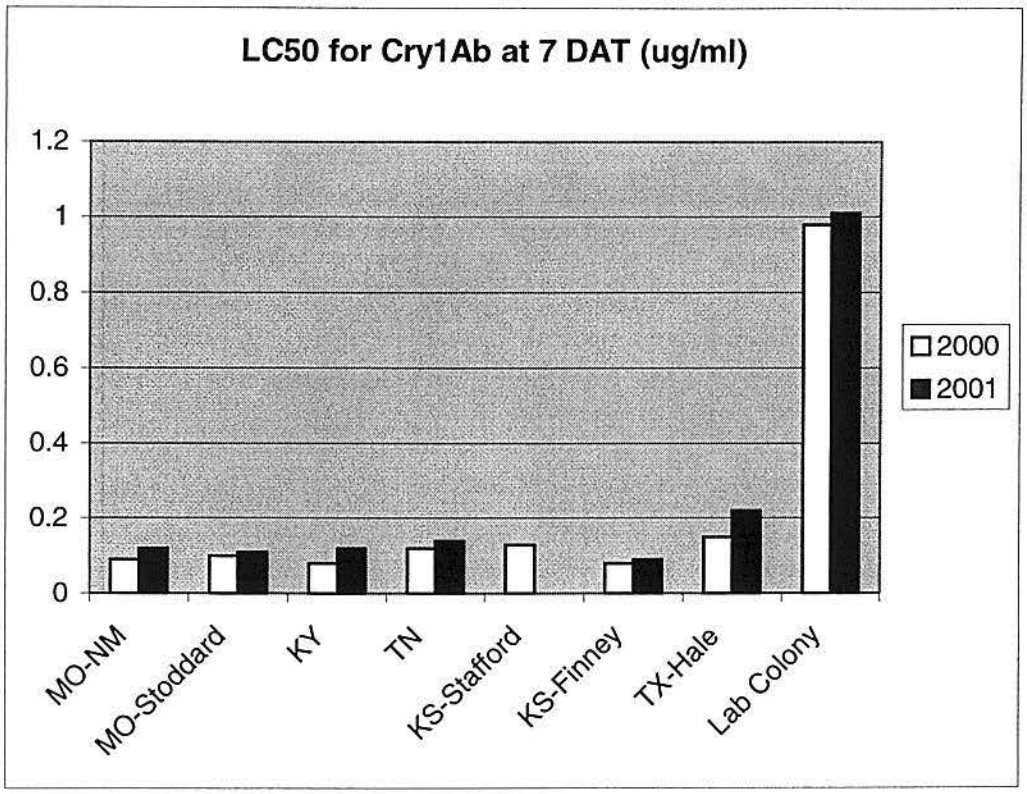
| Collection Site | $LC_{50}$ (95% CL) (µg/ml of diet) | $LC_{95}$ (95% CL) (µg/ml of diet) | % Mortality* (diagnostic) |
|---|---|---|---|
| **7 days after treatment** | | | |
| MO-New Madrid | 5.27 (3.32-13.45) | 51.0 (28.9-68.4) | 100 |
| MO-Stoddard | 4.87 (3.35-10.29) | 48.5 (35.3-82.8) | 100 |
| KY-Coldwell | 5.53 (2.93- 8.52) | 33.8 (21.1-59.7) | 100 |
| TE-Gibson | 4.43 (2.57- 6.72) | 38.7 (18.6-60.1) | 100 |
| KS-Stafford | No sample for 2001 | | |
| KS-Finney | 3.69 (1.93- 7.01) | 34.5 (19.2-48.7) | 100 |
| TX-Hale | 5.73 (3.09-12.16) | 63.3 (24.6-89.8) | 100 |
| Lab colony | 27.2 (17.6-39.81) | 209.0 (161.2-396.4) | 100 |
| **14 days after treatment** | | | |
| MO-New Madrid | 1.37 (1.02-3.69) | 16.21 (7.89-34.89) | 100 |
| MO-Stoddard | 1.61 (1.03-2.45) | 14.76 (5.80-33.65) | 100 |

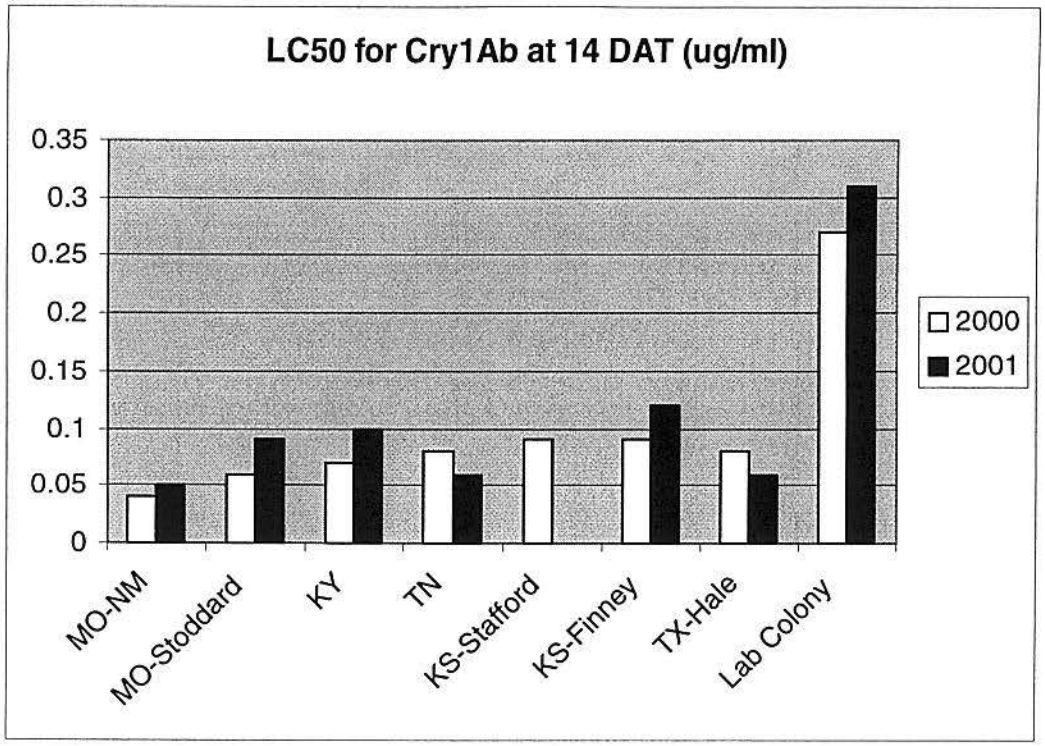
| | | | | |
|---|---|---|---|---|
| KY-Coldwell | 1.63 (0.43-2.91) | 16.34 (3.25-28.13) | | 100 |
| TE-Gibson | 0.87 (0.62-1.89) | 7.37 (3.34- 12.39) | 100 | |
| KS-Stafford | No sample for 2001 | | | |
| KS-Finney | 0.79 (0.51-0.98) | 6.26 (3.03- 8.98) | | 100 |
| TX-Hale | 1.59 (0.85-2.83) | 18.03 (8.06- 42.7) | | 100 |
| Lab colony | 7.05 (3.98-15.28) | 64.92 (37.5-147.9) | | 100 |

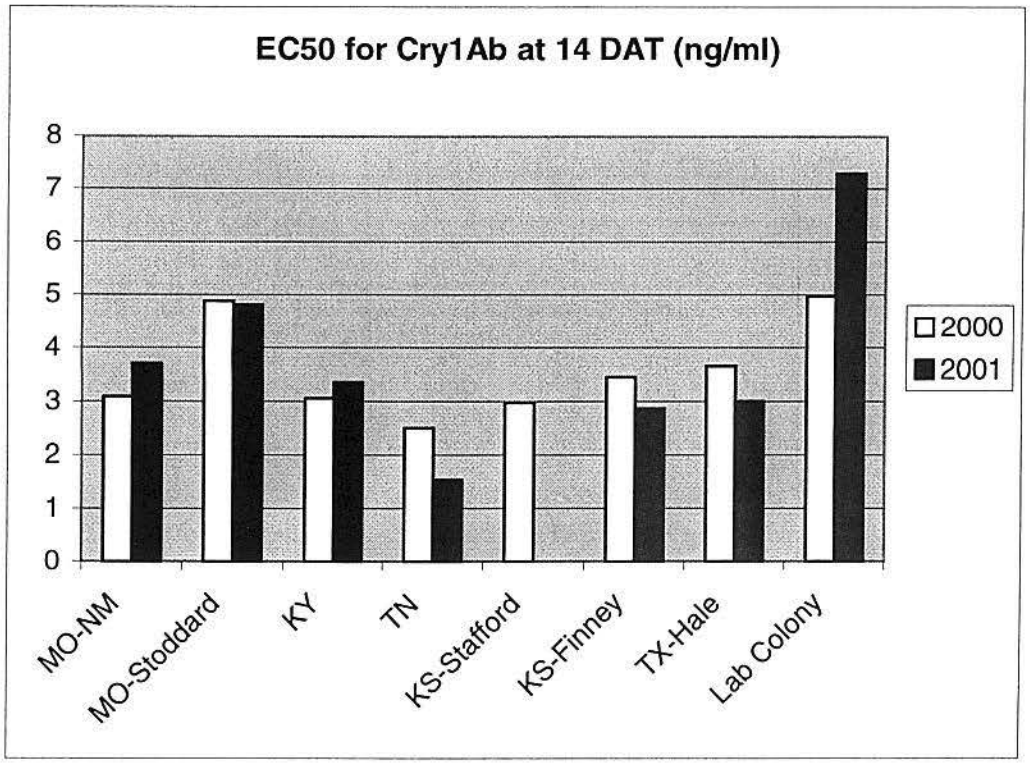
*: The $LC_{99}$ of the Cry1F Bt toxin was estimated from baseline susceptibility studies conducted on 8 populations in 2000, and 200 larvae from each field population were tested for the diagnostic concentration. The larvae were counted as dead if motionless when approached by a brush or if remaining in the first instar stage.

Table 3. Growth inhibition of neonate larvae fed continuously on an artificial diet containing Cry1F Bt toxin (14 days after treatment).

| Collection Site | $EC_{50}$ (95% CL) (µg/ml of diet) | $EC_{95}$ (95% CL) (µg/ml of diet) |
|---|---|---|
| MO-New Madrid | 0.072 (0.043-0.163) | 0.47 (0.28-0.86) |
| MO-Stoddard | 0.078 (0.054-0.245) | 0.59 (0.38-0.94) |
| KY-Coldwell | 0.072 (0.047-0.099) | 0.39 (0.28-0.57) |
| TE-Gibson | 0.063 (0.058-0.173) | 0.76 (0.39-1.06) |
| KS-Stafford | No sample for 2001 | |
| KS-Finney | 0.061 (0.037-0.096) | 0.57 (0.38-0.91) |
| TX-Hale | 0.087 (0.042-0.157) | 0.46 (0.23-0.88) |
| MS-Lab colony | 0.127 (0.087-0.267) | 1.61 (0.42-6.28) |


LC50 for Cry1Ab at 7 DAT (ug/ml)
1.2
1
0.8
0.6
0.4
0.2
0
2000
2001
MO-NM
MO-Stoddard
KY
TN
KS-Stafford
KS-Finney
TX-Hale
Lab Colony


LC50 for Cry1Ab at 14 DAT (ug/ml)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
MO-NM
MO-Stoddard
KY
TN
KS-Stafford
KS-Finney
TX-Hale
Lab Colony
2000
2001


EC50 for Cry1Ab at 14 DAT (ng/ml)
8
7
6
5
4
3
2
1
0
MO-NM
MO-Stoddard
KY
TN
KS-Stafford
KS-Finney
TX-Hale
Lab Colony
2000
2001

# MONITORING THE SUSCEPTIBILITY OF THE SOUTHWESTERN CORN BORER, *DIATRAEA GRANDIOSELLA*, TO *BACILLUS THURINGIENSIS* TOXIN Cry1F

## 2001 Data Summary

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University of Missouri
Columbia, MO 65211

(January 22, 2002)

## INTRODUCTION

This is a continuous effort to monitor the susceptibility of the Southwestern corer borer (SWCB), *Diatraea grandiosella*, to *Bacillus thuringiensis* (Bt.) toxin Cry1F.

In this report, six field populations of the SWCB were collected from two regions with high Bt-corn penetration rate and bioassayed to determine a baseline susceptibility (larval mortality, larval growth inhibition and response to diagnostic dose) to Bt. toxin Cry1F

## EXPERIMENT PROCEDURES

### a. Field SWCB sample collection

The SWCB field samples were collected from the same locations as we did last year (Table 1). In brief, two sample collection regions were selected based on the high Bt-corn penetration rate and/or a history of insecticide application in refuge areas. The region one includes southwest Kansas and the panhandle area of Oklahoma and Texas with both a high rate of Bt-corn and a history of insecticide applications. Two field populations were collected from this region. Diapause larvae (147) were collected from Garden City of KS by Larry Buschman of KSU and second flight Female adults (103)

were blacklight-trapped from Hale Co. of TX by Pat Porter of TAM. There was no sample available from St. John (KS), presumably due to extremely cold winter last year (personal communication with Larry Buschman). The Region two includes the areas of Missouri Bootheel, Western Kentucky, Western Tennessee, and Extreme Southern Illinois with a relatively high penetration of Bt. corn. Four field populations were collected from the region two (Michael Boyd of MU at Delta Center collected one population of diapause larvae from Delta center, MO; My student Chris Luppens and I collected three field populations by blacklight trapping, one each from MO-Dexter, TE-Milan, and KY-Princeton) (Table 1).

**b. Rearing of SWCB**

Larvae generated from the field-collected populations and from a MS-laboratory colony were maintained on a BioServe artificial diet in clear plastic cups (20 ml) using established laboratory procedures (Chippendale and Cassatt, 1985). Larvae are incubated at $30^0$C and 16 hr L : 8 hr D, whereas eggs, pupae, and adults are incubated at $25^0$C and 13 hr L : 11 hr D.

**c. CrylF**

Cry1F toxin was provided by Pioneer Hibred last year (7859.4 mg powder containing 1.3% Cry1F), aliquoted and stored at $-80^0$C until use.

**d. Susceptibility ($LC_{50}$ and $EC_{50}$) of SWCB neonate larvae to Cry1F toxin**

*Larval mortality*

A larval feeding bioassay was used. Cry1F toxin was diluted in a 50 ml centrifuge tube containing 1 ml of distilled water to make a serial of desired concentration, and the solution was incorporated into 40 ml of artificial diet (kept at $55^0$C water bath) by inverting and shaking the tube. The diet containing the indicated dose of Cry1F was then pipetted into a 128-cavity tray (BIO BA 128 bioassay tray, C-D International, Ocean City, NJ) with 1 ml of diet/cavity. The diet-Cry1F containing tray was either assayed shortly after cool down or kept at $4^0$C for < 48 h before use. Newly hatched larvae (<24 h) were transferred individually to each cavity containing

1 ml of diet with or without the indicated concentration of Cry1F. Each concentration was replicated four times (25 larvae replicate$^{-1}$) using larvae from different batches of the $F_2$ or $F_3$ generations (the number of $F_1$ generation larvae were usually not enough for the assays in such a scale). Larval mortality was observed at 7 and 14 days after treatment (DAT). Larvae are considered dead if they do not move when they are probed using a camel hairbrush.

Six or seven consecutive concentrations of each toxin, including the control, were used for probit analyses to determine the $LC_{50}$ and $LC_{95}$ values on 7 and 14 DAT. These concentrations produce larval mortality of >0% but <100%. Probit analyses were conducted, using SAS program (SAS institute Inc., Cary, NC).

*Larval growth inhibition*

Six concentrations of Cry1F were used in bioassay. Each concentration was replicated 4 times (25 larvae replicate$^{-1}$) from different batches as described above. The mean weight of newly hatch larvae (initial weight) was determined by weighing a group of 25 and the mean weight of those surviving larvae at each tested dose was determined at 7 and 14 DAT.

Regression analyses (log concentration vs. weight gain inhibition [%]), are used to determine the $EC_{50}$ and $EC_{95}$ values at 7 and 14 DAT. Weight gain inhibition represents the relative differences in weight gain of the treated larvae compared to that of the control larvae. The following equation is used to correct for initial weight: $I = \{(C - T) \div (C - B)\} \times 100\%$, where I = weight gain inhibition (%); C = mean weight of control larva (mg larva$^{-1}$); T = mean weight of surviving larvae from the treated diets (mg larva$^{-1}$); and B = mean initial larval weight (mg larva$^{-1}$).

*Efficacy of the diagnostic concentration against the laboratory-adapted and field-collected populations* of *Diatraea grandiosella*

The diagnostic concentration was estimated from the $LC_{99}$ value at 7 and 14 DAT of the MS-Laboratory population because this population has been found to be the least susceptible to Cry1F protein of the populations tested. 6 field populations of SWCB were used to test the efficacy of the diagnostic concentration (216.4 µg/g diet for 7 DAT, 68.6 µg/g diet for 14 DAT) in determining the susceptibility to Cry1F.

Two hundred of newly hatched larvae from $F_2$ or $F_3$ of each field population were

transferred individually in to the 128-cavity tray containing 1 ml of control diet or the diet with the diagnostic concentration of Cry1F. On the 7 and 14 DAT, larval mortality was recorded.

**Results and Discussions**

Bioassay of the susceptibility of neonate SWCB larvae to Cry1F toxin had been summarized in Table 2 and 3. This was the continuation of the previous year's effort to monitor the susceptibility of the SWCB to Bt. toxin Cry1F. All bioassays were performed in128-cavaty tray (C-D International) with 1 ml of diet containing the indicated concentrations of Cry1F toxin and at least 100 larvae were tested for each concentration of Cry1F.

Two bioassay procedures (larval mortality vs. growth inhibition) were employed to investigate the sensitivity and efficiency of Cry1F to SWCB. Based on the $LC_{50}$ (Table 2) and $EC_{50}$ (Table 3), significant variation [$LC_{50}$ from 3.69 to 5.73 µg/ml diet for 7 DAT and from 0.87 to1.63 µg/ml diet for 14 DAT (Table 2), $EC_{50}$ from 0.061 to 0.087 µg/ml diet for 14 DAT (Table 3)] was observed among geographically distinct populations of SWCB in their susceptibility to Cry1F toxin. However, all field-collected populations were more susceptible to Cry1F toxin than the MS-lab colony (MS-lab colony was reintroduced from Frank Davis's lab (MS) in Fall of 2000). The bioassay using growth inhibition is considered to be more sensitive in determining the susceptibility of the SWCB to Cry1F than using larval mortality. The slight increase in $LC_{50}$ and $EC_{50}$ values in both field populations and lab colony was observed when compared with the data of the previous year (Fig. 1), indicating the possibility of decreasing in toxicity of Cry1F, presumably due to handling and longtime storage process of the toxin (the Cry1F was from 2000 stock).

The diagnostic concentration was estimated from the $LC_{99}$ value of MS-lab colony, the least susceptible colony determined in feeding bioassay. Although a very few larvae survived the diagnostic dose, they remained at the size of first instar (the inoculated stage) and were considered as dead simply because that such larvae could not survive in natural environment. With that in mind, the mortality in diagnostic

concentration for all 6 field populations was 100% (Table 2) since the $LC_{99}$ value of MS-lab colony was much higher than that of the 7 field populations tested in 2000.

This is the second year that the susceptibility of SWCB to Cry1F was bioassayed in this lab. No significant changes in the susceptibility of SWCB to Cry1F were detected when the data from this year were compared to that of last year (Figure 1).

## REFERENCES


Chippendale GM and Cassatt KL (1985) *Diatraea grandiosella,* in *Handbook of Insect Rearing, vol 2,* ed Singh P and Moore RF, Elsevier Science Publishers, Amsterdam, pp 257-264.

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| | Kansas | Finney (10-19) | Garden City | 147 DL |
| | Texas | Hale (10-19) | Plainview | 103 FA ($F_2$) |
| Lab colony | Mississippi | Laboratory | MS-Lab | >1,000 EM |

* These two regions were selected for SWCB sample collection by ABSTC in March, 2000.
EM= egg masses; DL=diapause larvae; $F_2$= second fly adults.

Table 2. Susceptibility of SWCB neonate larvae to the Cry1F Bt toxin.

| Collection Site | $LC_{50}$ (95% CL) | $LC_{95}$ (95% CL) | % Mortality* |
|---|---|---|---|

| | (µg/ml of diet) | (µg/ml of diet) | (diagnostic) |
|---|---|---|---|
| **7 days after treatment** | | | |
| MO-New Madrid | 5.27 (3.32-13.45) | 51.0 (28.9-68.4) | 100 |
| MO-Stoddard | 4.87 (3.35-10.29) | 48.5 (35.3-82.8) | 100 |
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| | | | |
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Table 3. Growth inhibition of neonate larvae fed continuously on an artificial diet containing Cry1F Bt toxin (14 days after treatment).

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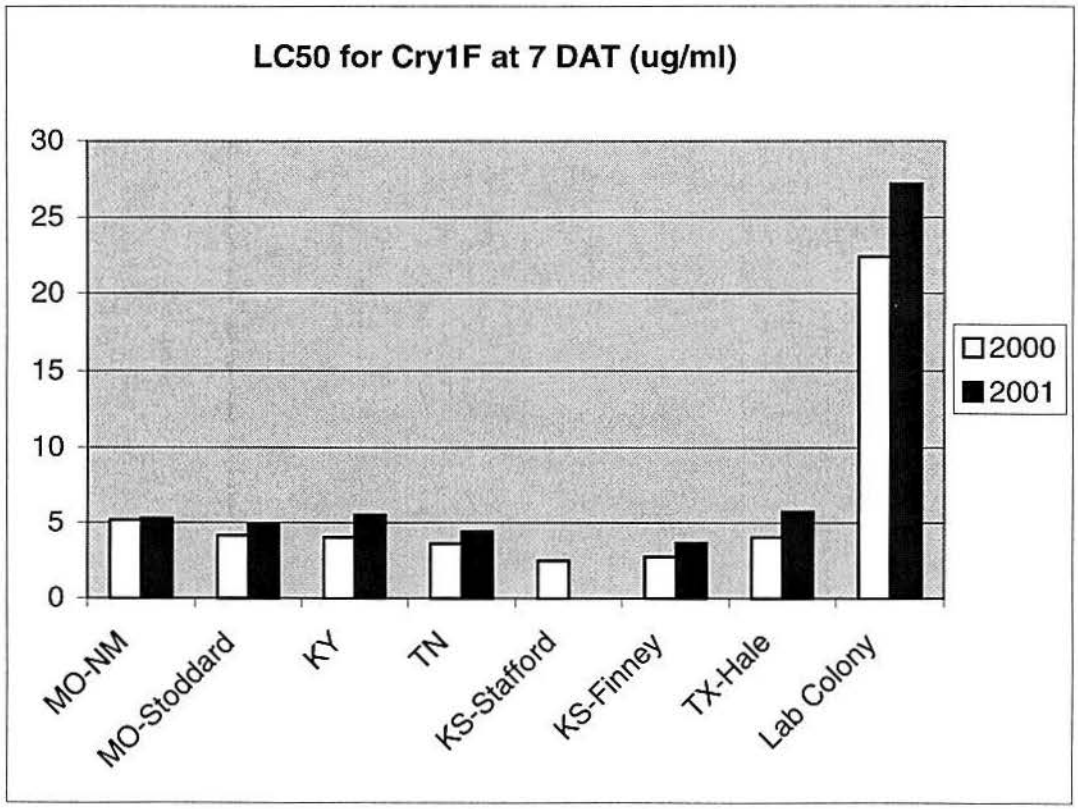
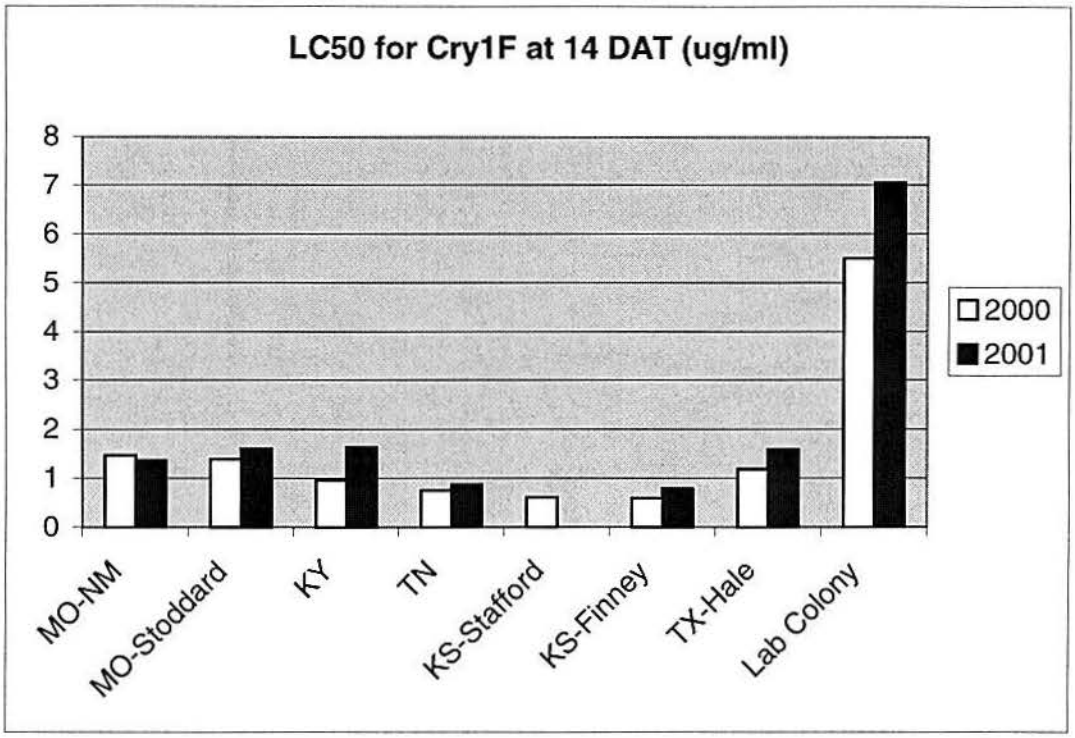
| MS-Lab colony | 0.127 (0.087-0.267) | 1.61 (0.42-6.28) |


LC50 for Cry1F at 7 DAT (ug/ml)
30
25
20
15
10
5
0
2000
2001
MO-NM
MO-Stoddard
KY
TN
KS-Stafford
KS-Finney
TX-Hale
Lab Colony


LC50 for Cry1F at 14 DAT (ug/ml)
8
7
6
5
4
3
2
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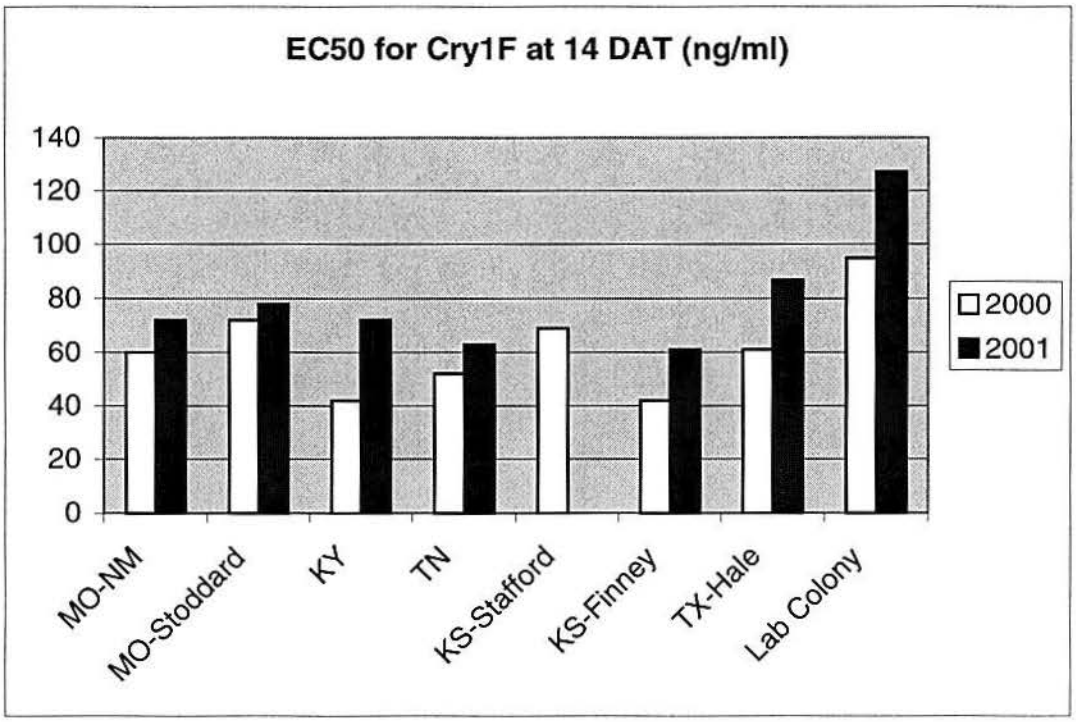
2000
2001
MO-NM
MO-Stoddard
KY
TN
KS-Stafford
KS-Finney
TX-Hale
Lab Colony


EC50 for Cry1F at 14 DAT (ng/ml)
140
120
100
80
60
40
20
0
MO-NM
MO-Stoddard
KY
TN
KS-Stafford
KS-Finney
TX-Hale
Lab Colony
2000
2001

# MONITORING Bt SUSCEPTIBILITY OF *H. zea* TO CRY1Ab

## 2002 Final Report
## March 26, 2002

### Custom Bio-Products

**INTRODUCTION:** Baseline susceptibility bioassays were conducted with the B.t. toxin Cry1Ab against 6 geographically distinct populations of corn earworm (*Helicoverpa zea*) which were collected from the U.S. corn and cotton belts. The bioassays were conducted using the protocols developed at the University of Nebraska (Siegfried et al. 2000).

**OBJECTIVE:** Compare levels of susceptibility to Cry1Ab among geographically distinct H. zea populations using dose-response regressions and diagnostic bioassays.

**METHODS:**

**Field Collections and Rearing:**

Field collections in the corn belt (IA, IL, and NE) were collected as late instar larvae in sweet corn and field corn ears from July through October, 2001. These larvae were placed on artificial diet and allowed to continue development through pupation.

Neonate larvae from 3 Virginia populations were obtained from Doug Sumerford at the USDA-ARS laboratory in Stoneville, MS.

Populations were reared using standard rearing protocols appropriate for this species. Eggs were collected from mated females and allowed to hatch. Neonate larvae were then used for bioassays or used to initiate another generation. All available neonate larvae up to 210 individuals per population per week were put into colony maintenance and production. Bioassays were initiated once the colonies became established.

2001 H. zea collections used for bioassay

| Collection Site | Date Obtained | Number of Larvae |
|---|---|---|
| Elkhart, Iowa | August 4 – 9 | 520 |
| Waterman, Illinois | October 2 | 517 |
| North Bend, Nebraska | July 28 – August 15 | 350 |
| Virginia (LC-3A) | September 17 | 70 |
| Virginia (LC-4A) | September 17 | 35 |
| Virginia (LC-5A) | September 17 | 30 |

**Bioassays:**

Nine dose response bioassays were run on f1 and f2 generations to find a dose range that would encompass all of the populations to give a range of response from little effect to <100% mortality. The diagnostic dose was estimated by using 2.5 times the highest level in the dose response bioassay.

Bioassay of neonate larvae involved exposure to B.t. solutions applied to the surface of single wells of artificial diet. The larvae used were from at least 2 generations from the wild. Bioassays were performed in 128 well trays (each well 16 mm diameter x 16 mm height; CD International, Pitman, NJ). Dilutions of B.t. were prepared in 0.1% Triton-X 100 to obtain uniform spreading of B.t. solution on the diet surface.

Individual neonate larvae (less than 24 h after hatching) were selected at random and placed in the treated wells, and mortality and individual surviving larval weight recorded 7 days later. Control treatments consisted of wells treated with 0.1% Triton-X 100. When recording mortality, larvae that had not grown equal to or greater than 10.0 mg were considered to be dead. Bioassays were conducted over time and across generations to run up to 6 reps per test per population. Each 128 well tray equals one replication. Within each rep of the dose response bioassay are 8 levels of B.t. exposure; 0 (control), 0.5, 1.0, 2.0, 4.0, 8.0, 12.0, and 16.0 ng B.t. protein / square cm surface area of diet. Each level consists of 16 individual wells. Each rep of the diagnostic bioassay consisted of 16 wells of 0.0 (control) and 112 wells of 40.0 ng B.t. protein / square cm surface area of diet. Data were analyzed by probit analysis (Finney 1971, LeOra Software 1987) to determine lethal concentrations. Observed mortality was corrected for mortality in control treatments, and lethal concentrations with 95% fiducial limits were calculated. Larval weights were transformed to % growth inhibition relative to the controls and these data were analyzed by non-linear regression (SAS Institute Inc. 1988).

The protein used for bioassays consisted of a formulated Cry1Ab provided by Dow AgroSciences (San Diego, CA; Lot# MR818 571-1457; 11.7 mg Cry1Ab / g formulation).

**RESULTS:**

The final test data were collected March 3rd, 2002 and colonies were shut down.

The enclosed tables will show analysis for EC50, EC95 & EC99's (TABLE 1) and LC50, LC90 & LC99's (TABLE 2). TABLE 3 lists % mortality data from diagnostic dose bioassays

**REFERENCES:**

Finney, D.J. 1971. Probit analysis. Cambridge University Press, England, 333 pp.

LeOra Software. 1987. POLO-PC. A user's guide to probit and logit analysis. Berkeley. CA.

SAS Institute Inc. 1988. SAS Procedures Guide, Release 6.03 Edition. Cary-NC. SAS Institute Inc., 441 pp.

Siegfried, B.D., T. Spencer, and J. Nearman. 2000. Baseline Susceptibility of the Corn Earworm (Lepidoptera: Noctuidae) to the Cry1Ab Toxin from *Bacillus thuringiensis*. Journal of Economic Entomology Vol. 93, no. 4.

## TABLE 1

## Susceptibility of Corn Ear Worm populations to Cry1Ab

| CRY1AB POPULATION | NUMBER OF REPS EVALUATED | $EC_{50}$(95% CL) NG A.I./$CM^2$ | $EC_{90}$(95% CL) NG A.I./$CM^2$ | $EC_{99}$(95% CL) NG A.I./$CM^2$ |
|---|---|---|---|---|
| **Illinois** | 6 | **0.15** (0.08 – 0.22) | **1.84** (1.43 – 2.51) | **28.30** (10.08 – 63.29) |
| **Iowa** | 4 | **0.31** (0.25 – 0.36) | **2.36** (1.94 – 2.91) | **21.59** (11.77 – 34.76) |
| **Nebraska** | 2 | **0.14** (0.06 – 0.22) | **1.56** (1.19 – 2.21) | **21.87** (6.82 – 53.81) |
| **#3 Virginia** | 6 | **0.16** (0.10 – 0.23) | **2.61** (2.02 – 3.54) | **53.98** (20.39 – 116.41) |
| **#4 Virginia** | 5 | **0.15** (0.10 – 0.20) | **1.28** (1.10 – 1.53) | **13.41** (7.02 – 22.97) |
| **#5 Virginia** | 6 | **0.18** (0.16 – 0.21) | **1.76** (1.60 – 1.96) | **20.73** (14.60 – 28.31) |

## TABLE 2

## Susceptibility of Corn Ear Worm populations to Cry1Ab

| CRY1AB POPULATION | NUMBER OF REPS EVALUATED | $LC_{50}$(95% CL) NG A.I./$CM^2$ | $LC_{90}$(95% CL) NG A.I./$CM^2$ | $LC_{99}$(95% CL) NG A.I./$CM^2$ | SLOPE | $CHI^2$ PROBABILITY |
|---|---|---|---|---|---|---|
| **Illinois** | 6 | **1.37** (0.54 – 2.32) | **9.30** (5.79 – 19.41) | **44.21** (20.75 – 211.64) | 1.54 + 0.17 | 0.89 |
| **Iowa** | 4 | **3.07** (2.26 – 3.81) | **8.22** (6.71 – 10.68) | **18.35** (13.50 – 29.85) | 2.99 + 0.40 | 0.17 |
| **Nebraska**[1] | 2 | **1.83** (0.32 – 3.64) | **9.49** (4.68 – 83.05) | **36.26** (12.50 – 3,488.37) | 1.79 + 0.36 | 0.77 |
| **#3 Virginia** | 6 | **4.98** (2.83 – 6.75) | **12.86** (9.98 – 17.87) | **27.86** (19.58 – 56.32) | 3.11 + 0.34 | 0.96 |
| **#4 Virginia** | 5 | **1.81** (1.41 – 2.21) | **6.21** (5.01 – 8.21) | **16.96** (12.02 – 28.12) | 2.39 + 0.26 | 0.54 |

## TABLE 3

## Mortality by colony exposed to Cry1Ab diagnostic concentration

| Colony | Rep | ng/cm sq | N | % Mortality |
|---|---|---|---|---|
| #3 Virginia | 1 | 40 | 112 | 100 |
| #3 Virginia | 2 | 40 | 112 | 100 |
| #3 Virginia | 3 | 40 | 112 | 100 |
| #3 Virginia | 4 | 40 | 112 | 100 |
| #3 Virginia | 5 | 40 | 112 | 100 |
| #3 Virginia | 6 | 40 | 112 | 100 |
| **#3 Virginia** | **All Reps** | **40** | **672** | **100** |
| | | | | |
| #4 Virginia | 1 | 40 | 112 | 100 |
| #4 Virginia | 2 | 40 | 112 | 100 |
| #4 Virginia | 3 | 40 | 112 | 100 |
| #4 Virginia | 4 | 40 | 112 | 100 |
| #4 Virginia | 5 | 40 | 112 | 100 |
| **#4 Virginia** | **All Reps** | **40** | **560** | **100** |
| | | | | |
| #5 Virginia | 1 | 40 | 112 | 96 |
| #5 Virginia | 2 | 40 | 112 | 99 |
| #5 Virginia | 3 | 40 | 112 | 100 |
| #5 Virginia | 4 | 40 | 112 | 100 |
| #5 Virginia | 5 | 40 | 112 | 100 |
| #5 Virginia | 6 | 40 | 112 | 100 |
| **#5 Virginia** | **All Reps** | **40** | **672** | **99** |
| | | | | |
| Iowa | 1 | 40 | 112 | 100 |
| Iowa | 2 | 40 | 112 | 100 |
| Iowa | 3 | 40 | 112 | 100 |
| Iowa | 4 | 40 | 112 | 100 |
| **Iowa** | **All Reps** | **40** | **448** | **100** |
| | | | | |
| Illinois | 1 | 40 | 112 | 99 |
| Illinois | 2 | 40 | 112 | 100 |
| Illinois | 3 | 40 | 112 | 100 |
| Illinois | 4 | 40 | 112 | 100 |
| Illinois | 5 | 40 | 112 | 100 |
| Illinois | 6 | 40 | 112 | 100 |
| **Illinois** | **All Reps** | **40** | **672** | **100** |
| | | | | |
| Nebraska | 1 | 40 | 112 | 100 |
| Nebraska | 2 | 40 | 112 | 100 |
| **Nebraska** | **All Reps** | **40** | **224** | **100** |

# MONITORING Bt SUSCEPTIBILITY OF H. zea TO CRY1Fa

## 2002 Final Report
## March 26, 2002

**Custom Bio-Products**

**INTRODUCTION:** Baseline susceptibility bioassays were conducted with the B.t. toxin Cry1Fa against 6 geographically distinct populations of corn earworm (*Helicoverpa zea*) which were collected from the U.S. corn and cotton belts. The bioassays were conducted using the protocol developed at the University of Nebraska (Siegfried et al. 2000).

**OBJECTIVE:** Compare levels of susceptibility to Cry1Fa among geographically distinct H. zea populations using dose-response regressions and diagnostic bioassays.

**METHODS:**

**Field Collections and Rearing:**

Field collections in the corn belt (IA, IL, and NE) were collected as late instar larvae in sweet corn and field corn ears from July through October, 2001. These larvae were placed on artificial diet and allowed to continue development through pupation.

Neonate larvae from 3 Virginia populations were obtained from Doug Sumerford at the USDA-ARS laboratory in Stoneville, MS.

Populations were reared using standard rearing protocols appropriate for this species. Eggs were collected from mated females and allowed to hatch. Neonate larvae were then used for bioassays or used to initiate another generation. All available neonate larvae up to 210 individuals per population per week were put into colony maintenance and production. Bioassays were initiated once the colonies became established.

2001 H. zea collections used for bioassay

| Collection Site | Date Obtained | Number of Larvae |
|---|---|---|
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| North Bend, Nebraska | July 28 – August 15 | 350 |
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| Virginia (LC-4A) | September 17 | 35 |
| Virginia (LC-5A) | September 17 | 30 |

**Bioassays:**

Several dose response bioassays were run on f1 and f2 generations to find a dose range that would encompass all of the populations to give a range of response from little effect to <100% mortality. The diagnostic dose was estimated by using 2.5 times the highest level in the dose response bioassay.

Bioassay of neonate larvae involved exposure to B.t. solutions applied to the surface of single wells of artificial diet. The larvae used were from at least 2 generations from the wild. Bioassays were performed in 128 well trays (each well 16 mm diameter x 16 mm height; CD International, Pitman, NJ). Dilutions of B.t. were prepared in 0.1% Triton-X 100 to obtain uniform spreading of B.t. solution on the diet surface.

Individual neonate larvae (less than 24 h after hatching) were selected at random and placed in the treated wells, and mortality and individual surviving larval weight recorded 7 days later. Control treatments consisted of wells treated with 0.1% Triton-X 100. When recording mortality, larvae that had not grown equal to or greater than 10.0 mg were considered to be dead. Bioassays were conducted over time and across generations to run up to 6 reps per test per population. Each 128 well tray equals one replication. Within each rep of the dose response bioassay are 8 levels of B.t. exposure; 0 (control), 25, 50, 100, 250, 500, 1000, and 2000 ng B.t. protein / square cm surface area of diet. Each level consists of 16 individual wells. Each rep of the diagnostic bioassay consisted of 16 wells of 0.0 (control) and 112 wells of 5000 ng B.t. protein / square cm surface area of diet. Data were analyzed by probit analysis (Finney 1971, LeOra Software 1987) to determine lethal concentrations. Observed mortality was corrected for mortality in control treatments, and lethal concentrations with 95% fiducial limits were calculated. Larval weights were transformed to % growth inhibition relative to the controls and these data were analyzed by non-linear regression (SAS Institute Inc. 1988).

The protein used for bioassays consisted of truncated Cry1Fa provided by Dow AgroSciences (Indianapolis, IN; Lot# 1599-45; 137 mg Cry1Fa / g formulation).

**RESULTS:**

The final test data were collected March 3rd, 2002 and colonies were shut down.

The enclosed tables will show analysis for EC50, EC95 & EC99's (TABLE 1) and LC50, LC90 & LC99's (TABLE 2). TABLE 3 lists % mortality data from diagnostic dose bioassays.

**REFERENCES:**

Finney, D.J. 1971. Probit analysis. Cambridge University Press, England, 333 pp.

LeOra Software. 1987. POLO-PC. A user's guide to probit and logit analysis. Berkeley. CA.

SAS Institute Inc. 1988. SAS Procedures Guide, Release 6.03 Edition. Cary-NC. SAS Institute Inc., 441 pp.

Siegfried, B.D., T. Spencer, and J. Nearman. 2000. Baseline Susceptibility of the Corn Earworm (Lepidoptera: Noctuidae) to the Cry1Ab Toxin from *Bacillus thuringiensis*. Journal of Economic Entomology Vol. 93, no. 4.

## TABLE 1

## Susceptibility of Corn Ear Worm populations to Cry1Fa

| CRY1F POPULATION | NUMBER OF REPS | $EC_{50}$(95% CL) NG A.I./$CM^2$ | $EC_{90}$(95% CL) NG A.I./$CM^2$ | $EC_{99}$(95% CL) NG A.I./$CM^2$ |
|---|---|---|---|---|
| **Illinois**[1] | 7 | **75.97** (54.42 – 101.96) | **857.90** (446.30 – 1,670.64) | **12,089.19** (2,004.62 – 34,157.91) |
| **Iowa** | 6 | **48.66** (43.50 – 53.98) | **618.15** (490.60 – 782.53) | **9,903.61** (5,654.52 – 15,511.69) |
| **Nebraska** | 2 | **229.14** (182.53 – 286.28) | **2,951.38** (1,579.40 – 4,217.38 ) | **36,573.08** (10,650.59 – 80,025.32) |
| **#3 Virginia** | 6 | **134.33** (91.50 – 191.82) | **1,783.35** (823.48 – 3,860.15) | **29,983.13** (3,695.07 – 96,427.19) |
| **#4 Virginia** | 2 | **43.17** (29.45 – 58.07) | **1,190.66** (637.74 – 2,331.36) | **44,457.12** (7,347.37 – 143,774.38) |
| **#5 Virginia** | 6 | **45.56** (40.18 – 51.09) | **711.89** (555.35 – 918.40) | **14,296.86** (7,741.42 – 23,370.62) |

[1]T1 included (formerly "rep. 0"). This provided significantly lower EC99 values.

## TABLE 2

## Susceptibility of Corn Ear Worm populations to Cry1F

| CRY1F POPULATION | NUMBER OF REPS | $LC_{50}$(95% CL) NG A.I./$CM^2$ | $LC_{90}$(95% CL) NG A.I./$CM^2$ | $LC_{99}$(95% CL) NG A.I./$CM^2$ | SLOPE | $CHI^2$ PROBABILITY |
|---|---|---|---|---|---|---|
| **Illinois** | 6 | **89.68** (36.64 – 165.78) | **1,062.98** (626.95 – 2,005.70) | **7,979.50** (3,789.80 – 25,645.00) | 1.19 + 0.07 | 0.99 |
| **Iowa** | 6 | **310.61** (120.46 – 545.90) | **2,137.04** (1,409.15 – 3,294.34) | **10,296.00** (6,061.00 – 24,627.00) | 1.53 + 0.15 | 0.80 |
| **Nebraska**[1] | 2 | **2,301.48** (1,703.38 – 2,769.84) | **4,372.22** (3,780.01 – 5,131.67) | **7,377.50** (6,104.80 – 10,061.00) | 4.60 + 0.71 | 0.49 |
| **#3 Virginia** | 6 | **1,075.64** (88.66 – 1,257.34) | **3,519.67** (3,127.67 – 4,001.14) | **9,251.80** (7,693.30 – 11,659.00) | 2.49 + 0.17 | 0.42 |
| **#4 Virginia** | 2 | **863.18** (546.65 – 1,119.78) | **1,810.27** (1,410.04 – 2,661.20) | **3,311.00** (2,343.60 – 7,021.30) | 3.98 + 0.87 | 0.12 |
| **#5 Virginia** | 6 | **492.45** (210.15 – 804.77) | **3,204.56** (2,245.04 – 4,950.42) | **14,754.00** (8,479.40 – 39,753.00) | 1.58 + 0.14 | 0.97 |

[1]Twenty-eight percent of substituted controls died in this dose test. Response fails to increase substantially until the 2000 ng. dose. These results were obtained only by eliminating some low dose responses and the actual control—while not leaving too few data points to generate results. These results (Nebraska) were obtained retaining some low dose responses which did not show any increased response between increasing low doses.

## TABLE 3

## Mortality by colony exposed to Cry1Fa diagnostic concentration

| Colony | Rep | ng/cm sq | N | % Mortality |
|---|---|---|---|---|
| #3 Virginia | 1 | 5000 | 112 | 98 |
| #3 Virginia | 2 | 5000 | 112 | 93 |
| #3 Virginia | 3 | 5000 | 112 | 94 |
| #3 Virginia | 4 | 5000 | 112 | 89 |
| #3 Virginia | 5 | 5000 | 112 | 99 |
| #3 Virginia | 6 | 5000 | 112 | 97 |
| **#3 Virginia** | **All Reps** | **5000** | **672** | **95** |
| | | | | |
| #4 Virginia | 1 | 5000 | 112 | 100 |
| #4 Virginia | 2 | 5000 | 112 | 100 |
| **#4 Virginia** | **All Reps** | **5000** | **224** | **100** |
| | | | | |
| #5 Virginia | 1 | 5000 | 112 | 94 |
| #5 Virginia | 2 | 5000 | 112 | 100 |
| #5 Virginia | 3 | 5000 | 112 | 92 |
| #5 Virginia | 4 | 5000 | 112 | 93 |
| #5 Virginia | 5 | 5000 | 112 | 97 |
| #5 Virginia | 6 | 5000 | 112 | 100 |
| **#5 Virginia** | **All Reps** | **5000** | **672** | **96** |
| | | | | |
| Iowa | 1 | 5000 | 112 | 100 |
| Iowa | 2 | 5000 | 112 | 100 |
| Iowa | 3 | 5000 | 112 | 100 |
| Iowa | 4 | 5000 | 112 | 94 |
| Iowa | 5 | 5000 | 112 | 96 |
| **Iowa** | **All Reps** | **5000** | **560** | **98** |
| | | | | |
| Illinois | 1 | 5000 | 112 | 100 |
| Illinois | 2 | 5000 | 112 | 99 |
| Illinois | 3 | 5000 | 112 | 98 |
| Illinois | 4 | 5000 | 112 | 98 |
| Illinois | 5 | 5000 | 112 | 97 |
| Illinois | 6 | 5000 | 112 | 97 |
| **Illinois** | **All Reps** | **5000** | **672** | **98** |
| | | | | |
| Nebraska | 1 | 5000 | 112 | 94 |
| Nebraska | 2 | 5000 | 112 | 97 |
| **Nebraska** | **All Reps** | **5000** | **224** | **95.5** |

DP BARCODE: D283430

CASE: 062714 DATA PACKAGE RECORD DATE: 06/05/02
SUBMISSION: S616181 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REGISTRATION ACTION: 570 CON REG FLW-UP DAT REQ RD
CHEMICALS: 006444 Bacillus thuringiensis subsp. kurstaki, delta-endo 0.0000%

ID#: 065268-00001 ATTRIBUTE INSECT PROTECTED SWEET CORN
COMPANY: 065268 SYNGENTA SEEDS, INC. - VEGETABLES - NAFTA
PRODUCT MANAGER: 90 JANET ANDERSEN 703-308-8128 ROOM: CS1 5TH FL
PM TEAM REVIEWER: MICHAEL MENDELSOHN 703-308-8715 ROOM: CS1 5TH FL
RECEIVED DATE: 05/01/02 DUE OUT DATE: 10/28/02

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 283430 EXPEDITE: N DATE SENT: 06/05/02 DATE RET.: / /
CHEMICAL: 006444 Bacillus thuringiensis subsp. kurstaki, delta-endotoxin as
DP TYPE: 001

| | CSF: | LABEL: | | |
|---|---|---|---|---|
| ASSIGNED TO | DATE IN | DATE OUT | ADMIN DUE DATE: 10/23/02 | |
| DIV : BPPD | / / | / / | NEGOT DATE: | / / |
| BRAN: BPPD-IO | / / | / / | PROJ DATE: | / / |
| SECT: IO | / / | / / | | |
| REVR : | / / | / / | | |
| CONTR: | | | | |
| | / / | / / | | |

* * * DATA REVIEW INSTRUCTIONS * * *

Attention IRM Team:

Please review the fall armyworm 2000 bioassay. Thanks.
MRID No. 456663-01

* * * DATA PACKAGE EVALUATION * * *

No evaluation is written for this data package

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

| DP BC | BRANCH/SECTION | DATE OUT | DUE BACK | INS | CSF LABEL |
|---|---|---|---|---|---|

456663-00

# TRANSMITTAL DOCUMENT

1. NAME AND ADDDRESS OF SUBMITTER:

   SYNGENTA SEEDS, INC.
   3054 CORNWALLIS ROAD
   POST OFFICE BOX 12257
   RESEARCH TRIANGLE PARK, NC 27709

2. REGULATORY ACTIONS IN SUPPORT OF WHICH THIS PACKAGE IS SUBMITTED:

   EPA REG. NO. 65268-1
   SUBMITTED IN FULLFILLMENT OF TERMS & CONDITIONS OF REGISTRATION OF THE FOLLOWING PLANT PESTICIDE ACTIVE INGREDIENT IN SWEET CORN

   *Bacillus thuringiensis* CryIAb delta-endotoxin and the genetic material (plasmid vector pZO1502) necessary for its production in corn

3. TRANSMITTAL DATE

   April 29, 2002

4. LIST OF SUBMITTED VOLUMES

| MRID NO. | VOL NO. | STUDY TITLE | EPA GUIDELINE NO. |
|---|---|---|---|
| 45666301 | 1 | Fall Armyworm Susceptibility to CryIA(b) toxin: 2000 Bioassay | N/A |

Company Official: David Guyer, Manager of Regulatory Affairs — Signature

Company Name: SYNGENTA SEEDS, INC.

Company Contact: David Guyer (Name) — (919) 541-8526 (Phone) — (919) 541-8535 (Fax)

456663-01

VOLUME 1 OF 1 SUBMISSION

SYNGENTA SEEDS, INC.

# FALL ARMYWORM SUSCEPTIBILITY TO CryIA(b) TOXIN: 2000 BIOASSAY

SUBMITTED IN FULFILLMENT OF TERMS & CONDITIONS OF REGISTRATION OF THE FOLLOWING PLANT-PESTICIDE ACTIVE INGREDIENT IN SWEET CORN:

*BACILLUS THURINGIENSIS* CRYIA(B) DELTA-ENDOTOXIN AND THE GENETIC MATERIAL (PLASMID VECTOR pZO1502) NECESSARY FOR ITS PRODUCTION IN CORN.

**EPA REG. NO. 65268-1**

EPA GUIDELINE #: NOT APPLICABLE

AUTHOR:

DAVID GUYER

SUBMITTED ON: April 29, 2002

SUBMITTED BY:

SYNGENTA SEEDS, INC.
REGULATORY AFFAIRS DEPT.
POST OFFICE BOX 12257
RESEARCH TRIANGLE PARK, NC 27709-2257

## STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained herein on the basis of falling within the scope of FIFRA §10(d) (1) (A), (B), or (C).

COMPANY: Syngenta Seeds, Inc.

COMPANY AGENT: David Guyer
Manager of Regulatory Affairs

DATE: 4/29/02

## STATEMENT CONCERNING

## GOOD LABORATORY PRACTICES

Good Laboratory Practice standards do not apply to the information contained herein; therefore, certification of Good Laboratory Practice compliance described in 40CFR part 160 is not applicable.

SUBMITTED BY

David Guyer
Manager of Regulatory Affairs
Syngenta Seeds

4/29/02
Date

SUBMITTED FOR: Syngenta Seeds, Inc.
Regulatory Affairs Dept.
Post Office Box 12257
Research Triangle Park, NC 27709-2257
USA

Report to Syngenta Seeds Inc.

# Fall Armyworm Susceptibility to CryIA(b) toxin: 2000 Bioassay

John J. Hamm, Robert E. Lynch, and Ron E. Myers

**Crop Protection and Management Research Laboratory**
**USDA-ARS**
**Tifton, GA**

Annual Report

**Introduction:**

Novartis Seeds, Inc. (Syngenta Seeds, Inc.), developed transgenic sweet corn with a modified *cryIA(b)* gene that confers a high level of resistance to the corn earworm, *Helicoverpa zea* (Boddie), and a moderately high level of resistance to the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (FAW) (Lynch and Wiseman 1999). Field research comparing transgenic *Bt* sweet corn with Bonus and Silver Queen using a minimal number of insecticide applications for management of injury to ears by the corn earworm and FAW (Lynch, Wiseman, and Sumner, 1999) also showed a high level of resistance to the corn earworm and FAW. With extreme insect pressure, injury to ears of transgenic *Bt* sweetcorn averaged only 7.9 $cm^2$ when no insecticide was applied, and damage was reduced to an average of only 1.7 $cm^2$ with five applications of methomyl. Silver Queen and Bonus averaged 323 and 168 $cm^2$ of injury, respectively, with no insecticide applications, and 172 and 50 $cm^2$, respectively, with five applications of methomyl. Injury to ears on *Bt* sweetcorn was not totally eliminated, but was minimal and confined to a few kernels at the ear tip.

EPA approved registration of Novartis' *Bt* sweet corn in 1998, but suggested that research be conducted to establish baseline susceptibility of FAW to the CryIA(b) toxin. We report here the third year of research on susceptibility of FAW collected from four locations in the South and Southwest to the CryIA(b) toxin.

**Materials and Methods:**

FAW larvae were collected from corn or sorghum at Homestead and Belle Glade, Florida, and from Weslaco and Corpus Christi, Texas, in April - June 2000 to establish laboratory colonies. A minimum of 200 larvae was collected from each location, placed individually on pinto bean diet, and shipped to the Crop Protection and Management Research Laboratory, Tifton, GA. Upon receipt, the larvae were placed in an incubator operated at 26.7°C, 75% RH, and a 16:8 light:dark photoperiod. Cups containing larvae were checked each Monday, Wednesday, and Friday for pupation and adult emergence. Upon emergence, adults were placed in a 15.6°C holding room until sufficient moths had emerged for mating. Approximately 25-40 pairs of moths were then placed in a 3.8 L carton lined with waxed paper and with a paper towel over the top for oviposition. The oviposition containers were placed in the incubator as noted above. Moths were fed a 10% sugar solution and the waxed paper and paper towel containing eggs were changed each Monday, Wednesday, and Friday. Upon hatching, 2-4 neonate larvae were placed in a cup containing pinto bean diet and the cup was capped, labeled with the FAW strain and date, and placed in the incubator to continue the individual colonies.

The CryIA(b) toxin provided by Novartis to conduct the bioassays was from a new isolation in 2001. Bioassays were conducted with larvae from colonies collected from the four locations noted above and compared with the Tifton Laboratory Colony whose larvae have never been exposed to the CryIA(b) toxin. One-day-old larvae reared on pinto bean diet were used for the bioassays. Pinto bean diet was dispensed into the 24 wells of a tissue culture plate (Nunclon, 12 mm diameter wells, 1.9 $cm^2$ surface area) and the diet was allowed to solidify. The CryIA(b) toxin was diluted in sterile deionized water and 0.1 ml applied to the surface of bean diet. The

solution was spread over the surface of the diet by rocking the tissue culture plate from side to side several times and then allowed to dry for approximately 1 hour. Concentrations evaluated were 0, 0.06, 0.18, 0.54, 1.62, 4.87, 14.62, and 43.86 $\mu g/cm^2$ surface area. After infesting each plate with 1 FAW/cell, a 10.2 x 15.2 cm piece of parafilm was placed over the wells, and a lid was placed on the tissue culture plate. A hole had previously been burned in the top of the culture plate with a No. 2 insect pin for each of the 24 cells. A No. 00 insect pin was then used to punch a hole through the parafilm by inserting the pin through each hole in the lid, which allowed for air/moisture exchange. The lids to the tissue culture plates were also secured with two No. 10 clip binders to prevent movement of larvae between cells. All plates with larvae were placed in an incubator operated at 26.7°C, 75% RH, and a 16:8 (light:dark) photoperiod. Mortality data were recorded at seven and ten days after treatment. The concentration-mortality response data were analyzed by probit analysis using the POLO-PC program (LeOra Software 1987).

**Results:**

Data for the analyses of mortality data at seven days after treatment with Cry1A(b) protein using one-day-old FAW larvae are presented in Table 1. Interestingly, the LC50 was greatest for larvae from the Tifton Laboratory colony which had never been exposed to Cry1A(b) toxin. The LC50 was lowest for larvae from the Weslaco colony, significant lower than for larvae from the Corpus Christi and Tifton Laboratory FAW colonies as evidenced by 95% confidence intervals that did not overlap. The LC50s for larvae from the Homestead and Belle Glade colonies also were significantly lower than for the Tifton Laboratory colony, and the LC50 for larvae from the Homestead colony was significantly lower than for the Corpus Christi colony. However, no significant differences were noted in the LC90's for all 5 colonies as the confidence intervals all overlapped. A test of the hypothesis that the slopes for the bioassays of all cultures was the same was rejected, primarily as a result of the difference in the susceptibility of the Tifton Laboratory and Corpus Christi colonies compared to those for the Belle Glade, Homestead, and Weslaco colonies. However, the test for parallelism was accepted, thus indicating that although the colonies may represent different populations, they were responding to the Cry1A(b) toxin in a similar manner.

Essentially the same results were noted in the bioassays read at 10 days (Table 2).

A comparison of data collected in 1998, i.e., before FAW were exposed to the Cry1A(b) toxin produced by *Bt* sweetcorn grown in the field, with that for 2000 is presented in Table 3. Larvae from all five FAW colonies were actually more susceptible to the Cry1A(b) toxin in the 2000 bioassay than in the 1998 bioassay conducted when *Bt* sweetcorn was first approved by EPA for commercial production. The 95% confidence intervals for the 1998 and 2000 LC50s for the Corpus Christi and Tifton Laboratory insect cultures overlapped, whereas those for the Belle Glade, Homestead, and Weslaco did not indicate that larvae from these cultures were actually more susceptible to the toxin in 2000 than they were in 1998. However, the 95% confidence intervals for the LC90s from 1998 and 2000 overlapped for all colonies with the exception of the Homestead culture. As noted previously, insects from the FAW larvae collected from Homestead, FL in 2000 were actually more susceptible to the Cry1A(b) toxin than larvae collected from this area in 1998.

A comparison of the ratios of the toxicity of Cry1A(b) in 1998 with those in 2000 for each colony (Table 3) also showed that the toxicities were very similar with less than a three-fold difference in both the LC50s and the LC90s. These data further substantiate the hypothesis that FAW are not developing resistance to the Cry1A(b) toxin.

In conclusion, bioassays conducted on FAW larvae collected from Belle Glade and Homestead, FL, and Corpus Christi and Weslaco, TX, in 1998, 1999, and 2000 and compared with FAW larvae from the Tifton Laboratory colony did not show an increase in the LD50 or LD90 which would indicate the development of resistance in these populations to the Cry1A(b) produced in the Novartis' *Bt* sweetcorn.

**References:**

Lynch, R. E., B. R. Wiseman, D. Plaisted, and D. Warnick. 1999. Evaluation of Transgenic Sweet Corn Hybrids Expressing CryIA(b) Toxin for Resistance to Corn Earworm and Fall Armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 92: 246-252.

Lynch, R. E., B. R. Wiseman, H. R. Sumner, D. Plaisted, and D. Warnick. 1999. Management of Corn Earworm and Fall Armyworm (Lepidoptera: Noctuidae) Injury on a Sweet Corn Hybrid Expressing a *cryIA(b)* Gene. J. Econ. Entomol. J. Econ. Entomol. 92: 1217-1222.

Table 1. Bioassay of CryIA(b) against 1-day-old larvae of *Spodoptera frugiperda* from colonies collected at 5 locations.

| Colony | Mortality at 7 days | | | | |
|---|---|---|---|---|---|
| | LC50 | | LC90 | | |
| | LC50 (µg/cm sq) | 95% CI | LC90 (µg/cm sq) | 95% CI | Slope ± SE |
| Tifton Laboratory | 1.03 | 0.76- 1.37 | 9.90 | 6.57 - 16.85 | 1.301 ± 0.085 |
| Belle Glade, FL | 0.44 | 0.28 - 0.66 | 5.44 | 3.37 - 10.45 | 1.176 ± 0.089 |
| Homestead, FL | 0.44 | 0.33 - 0.56 | 5.07 | 3.62 - 7.73 | 1.201 ± 0.087 |
| Corpus Christi, TX | 0.94 | 0.61 - 1.37 | 11.02 | 6.97 - 20.45 | 1.199 ± 0.096 |
| Weslaco, TX | 0.36 | 0.22 - 0.54 | 4.53 | 2.75 - 9.10 | 1.162 ± 0.089 |

Analysis based on 4 replications, 24 larvae/replication, and 7 concentrations plus a control.

Table 2. Bioassay of CryIA(b) against 1-day-old larvae of *Spodoptera frugiperda* from colonies collected at 5 locations.

| Colony | Mortality at 10 days | | | | |
|---|---|---|---|---|---|
| | LC50 | | LC90 | | |
| | LC50 (µg/cm sq) | 95% CI | LC90 (µg/cm sq) | 95% CI | Slope ± SE |
| Tifton Laboratory | 1.03 | 0.82- 1.29 | 8.63 | 6.26 - 12.82 | 1.389 ± 0.090 |
| Belle Glade, FL | 0.39 | 0.24 - 0.60 | 5.53 | 3.35 - 10.93 | 1.116 ± 0.087 |
| Homestead, FL | 0.38 | 0.29 - 0.48 | 4.28 | 3.06 - 6.48 | 1.214 ± 0.089 |
| Corpus Christi, TX | 0.86 | 0.55 - 1.26 | 9.77 | 6.14 - 18.40 | 1.215 ± 0.099 |
| Weslaco, TX | 0.27 | 0.16 - 0.41 | 3.21 | 1.97 -6.31 | 1.191 ± 0.095 |

Analysis based on 4 replications, 24 larvae/replication, and 7 concentrations plus a control.

Table 3. Comparison of LD50, LD90 for Cry1A(b) and confidence intervals for fall armyworm colonies collected from different geographical locations. in 1998 and 2000.

| Colony | **Mortality at 7 days** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LC50 | | | | | LC90 | | | | | Slope ± SE | |
| | $\mu g/cm^2$ | | | 95% CI | | $\mu g/cm^2$ | | | 95% CI | | | |
| | 1998 | 2000 | Ratio (98:00) | 1998 | 2000 | 1998 | 2000 | Ratio (98:00) | 1998 | 2000 | 1998 | 2000 |
| Tifton Laboratory | 1.74 | 1.03 | 1.69:1 | 1.24-2.49 | 0.76 - 1.37 | 21.10 | 9.90 | 2.13:1 | 11.96-48.47 | 6.57 - 16.85 | 1.18 ± 0.13 | 1.301 ± 0.085 |
| Belle Glade, FL | 1.11 | 0.44 | 2.52:1 | 0.69-1.61 | 0.28 - 0.66 | 8.58 | 5.44 | 1.58:1 | 5.52-16.39 | 3.37 - 10.45 | 1.44 ± 0.19 | 1.176 ± 0.089 |
| Homestead, FL | 1.29 | 0.44 | 2.93:1 | 0.93-1.78 | 0.33 - 0.56 | 13.12 | 5.07 | 2.59:1 | 8.01-26.37 | 3.62 - 7.73 | 1.27 ± 0.13 | 1.201 ± 0.087 |
| Corpus Christi, TX | 1.50 | 0.94 | 1.60:1 | 1.04-2.13 | 0.61 - 1.37 | 14.50 | 11.02 | 1.32:1 | 8.79-29.85 | 6.97 - 20.45 | 1.30 ± 0.15 | 1.199 ± 0 096 |
| Weslaco, TX | 0.90 | 0.36 | 2.50:1 | 0.60-1.26 | 0.22 - 0.54 | 6.13 | 4.53 | 1.35:1 | 4.08-10.83 | 2.75 - 9.10 | 1.54 ± 0.19 | 1.162 ± 0.089 |

Table 4. Comparison of LD50, LD90 for Cry1A(b) and confidence intervals for fall armyworm colonies collected from different geographical locations. in 1998, 1999 and 2000.

| Insect Colony | Mortality at 7 days | | | | | |
|---|---|---|---|---|---|---|
| | $\mu g/cm^2$ | | | 95% CI | | |
| | 1998 | 1999 | 2000 | 1998 | 1999 | 2000 |
| | **LC50** | | | | | |
| Tifton Laboratory | 1.74 | 9.71 | 1.03 | 1.24-2.49 | 6.35 - 14.41 | 0.76 - 1.37 |
| Belle Glade, FL | 1.11 | 6.59 | 0.44 | 0.69-1.61 | 4.75 - 8.95 | 0.28 -0.66 |
| Homestead, FL | 1.29 | 2.14 | 0.44 | 0.93-1.78 | 0.78 - 4.52 | 0.33 -0.56 |
| Corpus Christi, TX | 1.50 | 10.22 | 0.94 | 1.04-2.13 | 5.83 - 20.22 | 0.61 - 1.37 |
| Weslaco, TX | 0.90 | 5.53 | 0.36 | 0.60-1.26 | 2.75 9.16 | 0.22 -0.54 |
| | **LC90** | | | | | |
| Tifton Laboratory | 21.10 | 70.46 | 9.90 | 11.96-48.47 | 39.52 - 202 | 6.57 - 16.85 |
| Belle Glade, FL | 8.58 | 52.04 | 5.44 | 5.52-16.39 | 33.07 - 102 | 3.37 - 10.45 |
| Homestead, FL | 13.12 | 60.22 | 5.07 | 8.01-26.37 | 23.87 - 310 | 3.62 - 7.73 |
| Corpus Christi, TX | 14.50 | 219.0 | 11.02 | 8.79-29.85 | 79.26 - 1500 | 6.97 - 20.45 |
| Weslaco, TX | 6.13 | 41.47 | 4.53 | 4.08-10.83 | 22.08 - 147 | 2.75 - 9.10 |
| | **Slope ± SE** | | | | | |
| Tifton Laboratory | 1.18 ± 0.13 | 1.49 ± 0.17 | 1.301 ± 0.085 | | | |
| Belle Glade, FL | 1.44 ± 0.19 | 1.43 ± 0.14 | 1.176 ± 0.089 | | | |
| Homestead, FL | 1.27 ± 0.13 | 0.88 ± 0.10 | 1.201 ± 0.087 | | | |
| Corpus Christi, TX | 1.30 ± 0.15 | 0.96 ± 0.10 | 1.199 ± 0.096 | | | |
| Weslaco, TX | 1.54 ± 0.19 | 1.47 ± 0.17 | 1.162 ± 0.089 | | | |